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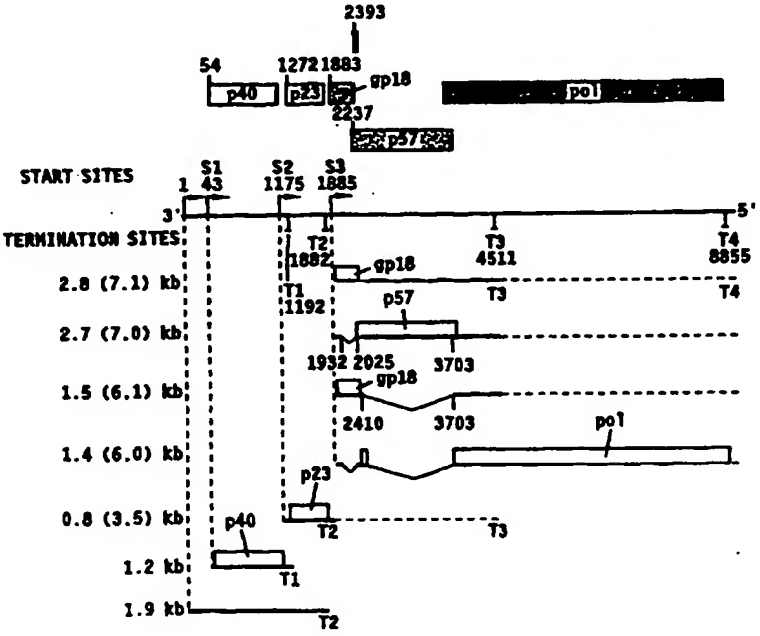

(57) Abstract

The present invention presents: genomic nucleotide sequence of Borna disease virus, nucleotide and amino acid sequences of Borna disease virus proteins, recombinant viral proteins, vectors and cells containing the sequences or encoding the proteins, ligand biding to these proteins such as antibodies, and the diagnostic and therapeutic uses of the foregoing.

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BORNA DISEASE VIRAL SEQUENCES, DIAGNOSTICS AND THERAPEUTICS FOR NERVOUS SYSTEM DISEASES

This patent application is a continuation-in-part of U. S. patent application Serial No. 08/434,831, filed on May 4, 1995, which is a continuation-in-part of U. S. patent application Serial No. 08/369,822, filed on January 6, 1995.

This invention was made with Government support under Grant No. NS29425, awarded by the National Institutes of Health (NINDS). The Government has certain rights in this invention.

FIELD OF THE INVENTION

The present invention relates to the field of virology, immunology, gene therapy, transplantation of viral transfected cells, and *in vivo* chemical delivery.

BACKGROUND OF THE INVENTION

Borna disease is an immune-mediated neurologic syndrome {Narayan, O., et al., *Science* 220:1401-1403 (1983)} caused by infection with Borna disease virus (BDV). BDV is a neurotropic, nonsegmented and negative-strand RNA virus that causes a progressive, immune-mediated neurologic disease characterized by disturbances in movement and behavior {Ludwig, H., et al., *Prog. Med. Virol*, 35:107-151}. It causes fatal disease in expensive domestic animals. Although natural infection was originally considered to be restricted to horses and sheep in Southeastern Germany, recent studies suggest that BDV infects horses in North America {Kao, M., et al., *Vet. Rec.*, 132:241-4 (1993)}, cats in Sweden {Lundgren,

A.-L., et al., *Zbl. Vet. Med. [B]*, 40:298-303 (1993)}, ostriches in Israel {Malkinson, M., et al., *Vet. Rec.*, 133:304 (1993)} and some human subjects with neuropsychiatric disorders in Europe and North America {Bode, L., et al., *Arch. Virol. [Suppl]*, 7:159-167 (1993); Bode, L., et al., *Lancet*, ii:689 (1988); Fu, Z. F., et al., *J. Affect. Disorders*, 27:61-68 (1993) and Rott, R., et al., *Science*, 228:755-756 (1985)}.

Experimental infection in rats {Narayan, O., et al., *Science*, 220:1401-1403 (1983)} results in a multiphasic syndrome characterized by hyperactivity, stereotyped behaviors, dyskinesias and dystonias.

Though natural infection has not been reported in primates, subhuman primates can be infected experimentally {Sprankel, H., et al., *Med. Microbiol. Immunol.* 165:1-18 (1978) and Stitz, L., et al., *J. Med. Virol.* 6:333-340 (1980)}. Antibodies to BDV proteins have been found in patients with neuropsychiatric disorders {Rott, R., et al., *Science* 228:755-756 (1985); Fu, Z. F., et al., *J. Affective Disord.* 27:61-68 (1993) and Bode, L., et al., *Arch. Virol. (Suppl.)* 7:159-167 (1993)}.

Because BDV grows only to low titer, it was difficult to purify for analysis. However, the identification of BDV cDNA clones by subtractive hybridization {Lipkin, W. I., et al., *Proc. Natl. Acad. Sci. USA* 87:4184-4188 (1990) and VandeWoude, S., et al., *Science* 250:1276-1281 (1990)} and, more recently, the advent of a method for isolation of virus particles {Briese, T., et al., *Proc. Natl. Acad. Sci. USA* 89:11486-11489 (1992)} led to partial characterization of BDV as a negative-strand RNA virus which transcribes its RNA in the cell nucleus {Briese, T., et al., *Proc. Natl. Acad. Sci. USA* 89:11486-11489 (1992)}.

The diagnosis of BDV infection is based on the appearance of a clinical syndrome consistent with

disease, and the presence of serum antibodies that detect viral proteins in infected cells by indirect immnunofluorescent test (IFT) {Pauli, G., et al., *Zbl. Vet. Med. [B]* 31:552-557 (1984)}, Western blot (WB; immunoblotting) or immunoprecipitation (IP) {Ludwig, H., et al., *Prog. Med. Virol*, 35:107-151 (1988)}. These methods are cumbersome and difficult to use for large surveys of human and livestock populations.

SUMMARY OF THE INVENTION

One aspect of the invention presents the nucleotide and amino acid sequences of Borna disease virus (BDV), their derivatives, the vectors for expressing them, and cells transfected by these vectors.

Another aspect of the invention presents novel BDV viral proteins gp18 and p57 and their respective recombinant proteins, recp18 and recp57. Also disclosed are their nucleotide and amino acid sequences, vectors encoding them, cells transfected by these vectors, and antibodies directed to these proteins.

Another aspect of the invention presents assays for detecting ligands which bind BDV proteins or their derivatives. Preferably, these assays are immunoassays for detecting antibodies to BDV protein or its derivatives. The assays are useful for detecting: (1) BDV infection or related pathogenesis; and (2) neurologic and neuropsychiatric disease not due to BDV infection. Preferably, p40, p23 or gp18, and their synthetic versions or fragments are used in these assays. The preferred immunoassays are enzyme-linked immunosorbent assays (ELISAs) based on the use of recombinant viral proteins: recp40, recp23, and/or recp18, and/or the immunoreactive fragments of the foregoing, to detect ligands, such as antibodies, in the patient's biological sample, that are immunoreactive with these proteins. The assay can also be used to monitor the diseases by

monitoring the titer of such ligands. The titer of the ligands can also be prognosticative of the diseases.

Another aspect of the invention presents alternative methods for detecting the above diseases by detecting the hybridization of nucleotide sequences in a patient's biological sample with the nucleotide sequences coding for BDV protein or its derivatives.

Another aspect of the invention presents assay kits for the above diagnostic tests.

Another aspect of the invention presents vaccines against the above diseases.

Another aspect of the invention presents synthetic peptides, based on truncated BDV protein, useful for immunoassays for detecting antibodies to BDV or for raising antibodies for the therapeutic uses described in the next paragraph. The method for obtaining these peptides are also presented.

Another aspect of the invention presents methods, using ligands or chemicals such as antibodies, capable of binding to BDV proteins or their derivatives, for treating: (1) BDV infection or related pathogenesis; and (2) neurologic and neuropsychiatric disease not due to BDV infection. Examples of such antibodies are those specific to gp18 and p57. Also presented are these therapeutic agents, methods for screening for them, especially those that bind to the immunogenic epitopes of BDV protein. The methods for producing the antibodies are also presented.

Another aspect of the invention presents a BDV-based viral vector useful for *in vivo* delivery of genes and chemicals to the nervous system. Also disclosed are: the cells transfected by the viral vector and cell lines derived therefrom, the *in vitro* harvesting of the gene product from such cells and cell lines, and the transplant of such cells into animals.

Other aspects and advantages of the invention will be apparent to those skilled in the art upon

consideration of the following detailed description which provides illustrations of the invention in its presently preferred embodiments.

Brief Description of the Drawings

FIG. 1 presents the genomic organization and transcriptional map of BDV.

FIG. 2 shows the complete genomic sequence of BDV (strain V) in 5' to 3' cDNA with the deduced amino acid sequence shown below the cDNA.

FIG. 3 (a) presents the organization of the BDV genome; (b) presents the coding potential of the genome.

FIG. 4 shows alignment of the p180 (also referred to as "pol") open reading frame (ORF) and negative-strand RNA virus L-polymerase amino acid sequences with PILEUP computer program (Sequence Analysis Software Package, Genetics Computer, Inc., Madison, Wisconsin). BDV sequence is indicated with double arrowheads. Rhabdoviridae: RaV, rabies virus; VSV, vesicular stomatitis virus; SYN, sonchus yellow net virus. Paramyxoviridae: MeV, measles virus; SeV, Sendai virus; NDV, Newcastle disease virus; RSV, respiratory syncytial virus. Filoviridae: MaV, Marburg virus.

FIG. 5 presents sequence analysis of BDV genomic termini. (a) Similarity of 3'-terminal BDV sequence to leader regions of Rhabdoviridae (RaV), VSV), Paramyxoviridae (MeV, SeV, NDV, RSV), and Filoviridae (MaV); (b) Comparison of complementarity at 3' and 5' termini of BDV genomic RNA with that of four other nonsegmented, negative-strand RNA viruses.

FIG. 6 presents the map of BDV subgenomic RNAs relative to the viral antigenome. (a) Northern hybridization analysis of rat brain poly(A)$^+$ RNA; (b) position of viral transcripts with respect to antigenome as determined by Northern hybridization and sequence analysis; (c) alignment of the seven potential termination sites of BDV.

FIG. 7 presents the sequence of ORF gp18.

FIG. 8 shows glycan determination of gp18. Lanes: 0, protein detection by mouse anti-gp18 serum; 1, ConA; 2, wheat germ agglutinin; 3, *D. stramonium* agglutinin; 4, BS-I; 5, BS-II; 6, *G. nivalis* agglutinin; 7, *S. nigra* agglutinin; 8, *M. amrensis* agglutinin; 9, peanut agglutinin. Positions of molecular weight markers are shown in kilodaltons at the right.

FIG. 9 presents treatment of gp18 with buffer alone or endoglycosidase. Lanes: 1, buffer; 2, endoglycosidase F plus *N*-glycosidase F; 3, endoglycosidase F (*N*-glycosidase free); 4, endo-β-galactosidase. Positions of molecular weight markers are shown in kilodaltons at the right.

FIG. 10 presents *in vitro* transcription, translation, and cotranslational processing of gp18. (A) Lanes: 1, pBDV-23 RNA; 2, pBDV-23 RNA plus microsomal membranes; 3, pBDV-gp18 RNA; 4, pBDV-gp18 RNA plus microsomal membranes; 5, pBDV-gp18 RNA plus microsomal membranes, incubated with endoglycosidases. (B) Lanes: 1, pBDV-gp18 RNA; 2, pBDV-gp18 RNA plus microsomal membranes; 3, pBDV-gp18 RNA plus microsomal membranes, incubated with endoglycosidases.

FIG. 11 presents Western blot analysis of native and recombinant proteins with monospecific antisera to recombinant proteins and sera from infected rats. (A) Lane 1, C6BDV lysate; lane 2, recp40; lane 3, recp23; lane 4, recp18; lane 5, C6 lysate; lane 6, recp40, recp23 and recp18. Lanes 1-4 were treated with serum from infected rat; lanes 5 and 6 were treated with serum from noninfected rat. (B) C6BDV lysates (lanes 1-3) and C6 lysates (lanes 4 and 5) were incubated with: lanes 1 and 4, serum from infected rat; lane 2, anti-p40 rabbit serum; lane 3, anti-p23 rabbit serum; and lane 5, pooled anti-p40 and anti-p23 sera.

FIG. 12 presents ELISA of infected rat serum reacted with recp40. Circles, recp40 and serum from chronically infected rat; squares, recp40 and serum from noninfected rat; triangles, BSA and serum from chronically infected rat.

FIG. 13 presents timecourse for the appearance of antibodies to BDV-proteins. (A) recp40; (B) recp23; and (C) recp18.

FIG. 14 presents timecourse for the appearance of antibodies to BDV proteins in sera from individual rats after intranasal infection. (A) Neutralization activity in sera from BDV-infected rats at three timepoints (5, 10 and 15 weeks post-infection). (B) Plot of mean recp18 ELISA titers (open columns) with neutralization titers (hatched columns) at three time points (5, 10 and 15 weeks post-infection). Sera analyzed were the same as those in panel A. (C) Timecourse for the appearance of antibodies to recp40, recp23, and gp18 by Western blot analysis.

FIG. 15 presents (A) Immunoprecipitation of gp18 with monoclonal antibodies (Mabs). Lanes: 1, serum from infected rat (15 week pi); 2, serum from noninfected rat; 3, MAb 14/29A5; 4, MAb 14/26B9; 5, MAb 14/8E1; 6, MAb 14/13E10; 7, MAb 14/18H7; 8, MAb 24/36F1 (MAb directed against the BDV 23 kDa protein, negative control); 9, no antibody. (B) MAbs were analyzed for binding to native gp18 in Western blot. Lanes: 1, serum from infected rat (15 week p.i., D2); 2, serum from noninfected rat; 3, MAb 14/29A5; 4, MAb 14/26B9; 5, MAb 14/8E1; 6, MAb 14/13E10; 7, MAb 14/18H7; and 8, MAb 24/36F1 (MAb directed against the BDV 23 kDa protein, negative control).

FIG. 16 presents neutralization profile of sera and MAbs. (A) Serum from noninfected rat. (B) serum from infected rat (15 week p.i., D2). (C) MAb 14/13E10. (D) MAb 14/29A5.

FIG. 17 presents precipitation of BDV with sera from infected rats. (A) Lanes: 1, serum from infected rat, 15

week p.i.; 2, serum from infected rat, 5 week p.i.; 3, serum from infected rat, 15 week p.i., no BDV; 4, serum from infected rat,15 week p.i., genome sense primer used for first strand cDNA synthesis. (B) Precipitation of BDV by monospecific antisera to recp18 and MAbs to gp18. Lanes: 1, monospecific rat antisera to recp18; 2, MAb 14/13E10; 3, MAb 14/29A5. DNA markers (basepairs) are shown at the right.

FIG. 18 presents the cDNA of BDV polymerase. "V" denotes the site of its intron which is located between nucleotide positions 2410 and 3703 in the figure. "I-2", denotes that this is the second intron in the BDV genome.

FIG. 19 presents the partial cDNA genomic sequence for BDV strain HE/80.

FIG. 20 graphically presents in A) the immunoreaction of truncated recp23 protein fragments with sera from 7 human schizophrenics (SZ Human), 4 BDV infected horses (BD Horse) and 6 BDV infected rats (BD Rat); and in B) the truncated recp23 fragments.

FIG. 21 graphically presents in A) the immunoreaction of truncated unglycosylated recp18 protein fragments with sera from 7 human schizophrenics (SZ Human), 6 BDV infected rats (BD Rat) and 2 mice immunized with native gp18 (Mouse α gp18); and in B) the truncated unglycosylated recp18 fragments.

FIG. 22 graphically presents the overlapping 8-mer peptides, derived from p23, lined up diagonally from the amino (left) terminus to the carboxyl (right) terminus. Above the overlapping peptides are blocks indicating the immunoreactive regions of p23 and presenting the mapped epitopes and their sequences.

FIG. 23 graphically presents the overlapping 8-mer peptides, derived from unglycosylated recp18, lined up diagonally from the amino (left) terminus to the carboxyl (right) terminus. Above the overlapping peptides are blocks indicating the immunoreactive regions of

unglycosylated gp18 and presenting the mapped epitopes and their sequences.

FIG. 24 graphically presents A) the SPOTs tests; B) the locations of immunoepitopes along the length of unglycosylated gp18 which are immunoreactive with the sera in the SPOTs tests of FIG. 24A. The sequences of the most immunoreactive epitopes are shown. The scale indicates by the darkness of the spots, the degree of immunoreaction. The lightest shade (Scale 1) indicates no detectable immunoreactivity; the darkest shade (Scale 4) indicates highest immunoreactivity.

FIG. 25 presents the predicted amino acid sequence and potential N-glycosylation sites of the BDV G-protein.

DETAILED DESCRIPTION OF THE INVENTION

BDV Protein, its Amino Acid and Nucleotide Sequences

Table 1 identifies the sequence ID Nos. with their respective nucleotide and amino acid sequences.

Table 1
Nucleotide and Amino Acid Sequences of Borna Disease Virus (BDV)

Nucleotide Sequence	Sequence ID No.
p40	1
p23	3
gp 18	5
p57	7
BDV polymerase	9
BDV genomic cDNA	19

Amino Acid Sequence	Sequence ID No.
p40	2
p23	4
gp 18	6
p57	8
BDV polymerase	10

BDV polymerase is also referred to as "pol" or "p180".

The present application discloses the complete BDV genomic nucleotide sequence, the locations on the genomic

nucleotide sequence which encode the different BDV proteins, the sites of splicing and overlap (see FIGs. 1 and 2). Also disclosed are the novel nucleotide and amino acid sequences of BDV proteins gp18, pol and p57. The following Figures 1, 2, 19, and Table 1 summarize this information.

FIG. 1 shows the genomic organization and transcriptional map of BDV. The BDV genome is shown as a solid line in 3' to 5' direction. Coding regions and their respective reading frames are represented as boxes at the top; the number above each upward vertical line indicates the nucleotide position of the first AUG codon in the respective ORF. Transcription initiation sites and their nucleotide positions on the viral genome (BDV strain V) are represented by arrows pointing downstream. Transcription termination sites and splice sites are indicated by downward vertical lines. Dashed lines indicate that readthrough at termination sites T2 and T3 results in synthesis of longer RNAs terminating at T3 and T4, respectively. The 1.2 kb and 0.8 kb RNA have been shown to represent the mRNAs for p40 and p23, respectively. p23 could also be translated from the 3.5 kb RNA. Transcripts that are likely to represent mRNAs for gp18, p57 and pol are indicated. Note that gp18 can only be translated from RNAs containing intron 1. Splicing of intron 1 preserves the gp18 initiation codon but introduces a stop codon such that only the first 13 amino acids could be translated from the 2.7 (7.0) kb transcripts and the RNA or the 1.4 kb RNA serve as messages for the translation of BDV proteins.

FIG. 2 shows the complete genomic sequence of BDV (strain V) in 5' to 3' cDNA. The deduced amino acid sequences are shown for p40, p23, gp18, p57 and pol. Note: the full amino acid sequence for pol after splicing modification is shown in sequence ID No. 10. The stars (*) indicate stop codons. Information on transcription and splicing of the genomic sequence is

found in Schneider, P.A. *et al., J. Virol.*, 68:5007-5012 (1994) and Schneemann, A., *et al., J. Virol.*, 68:6514-6522 (1994), both references are hereby incorporated by reference in their entirety.

FIG. 19 presents the partial cDNA genomic sequence (also listed as SEQ ID No. 33) of BDV strain HE/80. Position 1 to 2651 of this sequence corresponds to position 1397 through 4054 of the cDNA genomic sequence of BDV strain V. The cDNA sequence of BDV strain HE/80 disclosed herein encodes part of the p23 and BDV polymerase proteins, and the complete gp18 and p57 proteins.

The term "nucleotide sequence" as used herein, unless otherwise modified, includes both ribonucleic acid (RNA) and deoxyribonucleic acid (DNA).

The sequences in Table 1 include both native and synthetic sequences. Unless otherwise modified, the term "protein" as used herein encompasses both native and synthetic polypeptide and peptide. Synthetic protein includes recombinant and chemically synthesized protein. Unless otherwise indicated, "gp18", "p57", and "pol" proteins include both their native and synthetic versions. "recp18", "recp57" and "recpol" are recombinant proteins of "gp18", "p57", and "pol" proteins, respectively. The terms "p57" and "recp57" herein include both the predicted protein of about 57 kDa, and the glycoprotein of about 94 kDa (G-protein), further described below.

Some of the nucleotide sequences disclosed are in the form of DNA. For example, SEQ ID No. 19 presents the BDV viral genomic sequence as cDNA of BDV viral genomic RNA. One skilled in the art would realize that the BDV viral genomic RNA is complementary to its cDNA that is shown in Figure 2. The term "BDV genomic nucleotide sequence" thus includes both the full cDNA and RNA sequences of the BDV genome. Further, as used in this application and claims, the SEQ ID Nos. and disclosed

sequences include: (1) the DNA sequences as disclosed, (2) the complementary nucleotide sequences (which may be RNA or DNA) to the disclosed sequences, (3) the corresponding RNA sequences to the listed DNA sequences wherein the Thymidine ("T") in the disclosed DNA sequences is replaced with Uracil ("U"), (4) nucleotide sequences wherein other nucleotides known in the art such as nucleotide analogs, replace those in the foregoing sequences, for example, 5-methyl-cytosine replacing cytosine, and (5) nucleotide sequences that are within a variance (with regard to the respective SEQ ID Nos. or disclosed nucleotide sequences) of at least about: 10%, preferably 28%, more preferably 30%, and most preferably 35%. For example, Kishi, M., *et al.*, "Sequence Variability of Borna Disease Virus Open Reading Frame II Found in Human Peripheral Blood Mononuclear Cells", *J. Virol.*, 70(1):635-640 (Jan. 1996), cloned, sequenced, and analyzed cDNA of BDV ORF-II which encodes p24, from the peripheral blood mononuclear cells of three psychiatric patients. Fifteen clones were studied. Intrapatient divergences of the BDV ORF-II nucleotide sequence were 4.2% to 7.3%, 4.8% to 7.3%, and 2.8% to 7.1% of the three patients, leading to differences of 7.7% to 14.5%, 10.3% to 17.1%, and 6.0% to 16.2%, respectively, in the deduced amino acid sequence for BDV p24. Interpatient divergencies among the 15 clones were 5.9% to 12.7% at the nucleotide level and 12.8% to 28.2% at the amino acids level. The nucleotide sequences of the 15 human BDV ORF-II cDNA clones differed from those of horse strains V and He/80-1 by 4.2% to 9.3%. This reference is hereby incorporated by reference in its entirety. The above discussion would analogously apply to RNA sequences disclosed in this application.

Since nucleotide codons are redundant, also within the scope of this invention are equivalent nucleotide sequences which include: nucleotide sequences which code for the same proteins or equivalent proteins. Thus,

nucleotide sequences which encode substantially the same or functionally equivalent amino acid sequence may be used in the practice of the invention.

The terms "BDV genomic nucleotide sequence", "p18", "recp18", "pol", "recpol", "p57", "recp57", as used in relation to nucleotide sequences are defined above, together with: (1) nucleotide sequences that are within a variance (with regard to the respective nucleotide sequences in Table 1) of at least about: 10%, preferably 28%, more preferably 30%, and most preferably 35% (see also the discussion of Kishi, M., *et al. J. Virol.*, **70(1)**, above); (2) nucleotide sequences that are capable of hybridizing to the coding sequences of the respective nucleotide sequences, under stringent hybridization conditions, (3) nucleotide sequences coding for gp18, recp18, p57, recp57, pol, and recpol proteins, and amino acid sequences of SEQ ID Nos. 6, 8, and 10 respectively; and (4) fragments of SEQ ID Nos. 6; 8; 10; nucleotide number 1 through 53 and nucleotide number 1880 through 8910 of SEQ ID NO 19 and their fragments; or other nucleotide sequences which, for example, encode proteins having substantially the same biological characteristics/activities of gp18, recp18, p57, recp57, pol, recpol proteins, respectively. Preferably, the determinative biological characteristic/activity is the retention of at least one immunoepitope. Preferably, when used in an immunoassay for BDV, these proteins are immunoreactive with antibodies directed to BDV but not detectably immunoreactive with non-BDV specific antibodies found in a biological sample such as a serum sample. Alternatively, the nucleotide sequences can be nucleotide probes of at least 10 nucleotides in length. Preferably, when used in a hybridization assay for BDV, these probes do not detectably hybridize to the nucleotide sequences of non-BDV organisms which are found in a biological sample such as a serum sample. Alternatively, the nucleotide sequences hybridize to at

least 10 consecutive nucleotides in the coding sequences of the above listed nucleotide sequences. The nucleotide sequences include a nucleotide sequence which encodes a protein containing at least 8; more preferably, 5 to 6; and most preferably, 4 amino acids. Preferably, the protein is specific to BDV or retain one or more biological functions of BDV. Examples of such biological functions are: BDV's ability to bind a particular cellular receptor, BDV's ability to target its host cells (*e.g.* cells and tissues of the nervous system, bone marrow, peripheral blood, mononuclear cells or brain), and BDV's effects on the functions of cells infected by it. The discussion herein similarly applies to p23, recp23, p80, recp80 nucleotide sequences, and the cDNA nucleotide sequence of FIG. 19, *e.g.* in reference to their respective SEQ ID NOs and FIGg. 19.

The terms "gp18", "recp18", "p57", "recp57", "pol", and "recpol", as used in relation to proteins are, respectively, as defined above together with: (1) protein variants containing amino acid sequences that are within a variance (with regard to the amino acid sequences of SEQ ID Nos. 6, 8, and 10, respectively) of at least about: 5%, preferably 28%, more preferably 30%, and most preferably 35% (see also the discussion of Kishi, M., *et al. J. Virol.*, **70(1)**, above); (2) the functional equivalents of these proteins and their variants, respectively; and (3) the derivatives, including fragments, of gp18, recp18, p57, recp57, pol, recpol, proteins and their variants, respectively. Preferably, when used in an immunoassay for BDV, these proteins are immunoreactive with antibodies directed to BDV but not detectably immunoreactive with non-BDV specific antibodies found in a biological sample such as a serum sample. Alternatively, these proteins each contains at least 8; more preferably, 5 to 6; and most preferably, 4 amino acids. Preferably, the latter proteins are specific to BDV or retain one or more biological

functions of BDV. Examples of such biological functions are: BDV's ability to bind a particular cellular receptor, BDV's ability to target its host cells (*e.g.* cells and tissues of the nervous system, bone marrow, peripheral blood, mononuclear cells or brain), and BDV's effects on the functions of cells infected by it. The discussion herein similarly applies to p23, recp23, p80, and recp80 proteins, *e.g.* in reference to their respective SEQ ID NOs.

Within the definition of "BDV" are BDV isotypes, strains, and BDV related viruses. The term "BDV proteins and their derivatives", includes BDV proteins, fragments of BDV proteins, proteins containing immunoepitopes of BDV, variants and functional equivalents of the foregoing. gp18 and p57 are examples of BDV proteins. Preferably, the immunoepitope is specific to BDV.

The variants can result from, *e.g.* substitution, insertion, or deletion of the amino acid sequences shown in Table 1. The derivatives of the proteins and their variants, include fragments of these proteins and their immunogenic epitopes. Preferably, each of the fragments contains at least one immunogenic epitope of BDV. More preferably, the fragment is capable of being bound by polyclonal antibodies directed to BDV. In the case of antibodies which recognize linear epitopes, they generally bind to epitopes defined by about 3 to 10 amino acids. Preferably, too, each variant retains at least one immunoepitope of BDV. Preferably the immunoepitope is specific to BDV.

Two amino acid sequences are functionally equivalent if they have substantially the same biological activities. The proteins may be fused to other proteins, for example, signal sequence fusions may be employed in order to more expeditiously direct the secretion of the BDV protein. The heterologous signal replaces the native BDV signal, and when the resulting fusion is recognized, *i.e.* processed and cleaved by the host cell, the BDV

protein is secreted. Signals are selected based on the intended host cell, and may include bacterial, yeast, insect, mammalian, and viral sequences. For example, the native BDV signal or the herpes gD glycoprotein signal is suitable for use in mammalian expression systems.

Substitutional variants of the proteins disclosed herein are those in which at least one residue in the disclosed sequences has been removed and a different residue inserted in its place. Preferably, the amino acid change is conservative. For example, such substitutions generally are made in accordance with the following Table 2.

TABLE 2

Original Residue	Exemplary Substitutions
Ala	ser
Arg	lys
Asn	gln; his
Asp	glu
Cys	ser; ala
Gln	asn
Glu	asp
Gly	pro
His	asn; gln
Ile	leu; val
Leu	ile; val
Lys	arg; gln; glu
Met	leu; ile
Phe	met; leu; tyr
Ser	thr
Thr	ser
Trp	tyr
Tyr	trp; phe
Val	ile; leu

Novel amino acid sequences, as well as isosteric analogs (amino acid or otherwise), are included within the scope of this invention.

A variant typically is made by site specific mutagenesis of the encoding nucleic acid, expression of the variant nucleic acid in recombinant cell culture and, optionally, purification from the cell culture for example by immunoaffinity adsorption on a column to which are bound polyclonal antibodies directed against the original protein from which the variant is derived.

Another class of variants are deletional variants. Deletions are characterized by the removal of one or more amino acid residues from the original protein sequence. Typically, deletions are used to affect the original protein's biological activities. However, deletions which preserve the biological activity or immune cross-reactivity of the original protein are preferred.

Deletions of cysteine or other labile residues also may be desirable, for example in increasing the oxidative stability of the original protein. Deletion or substitutions of potential proteolysis sites, *e.g.* Arg Arg,

is accomplished by deleting one of the basic residues or substituting one by glutaminyl or histidyl residues.

It will be understood that some variants may exhibit reduced or no biological activity. These variants nonetheless are useful as standards in immunoassays for BDV protein so long as they retain at least one immunogenic epitope of BDV protein.

It is presently believed that the three-dimensional structure of the proteins of the present invention is important to their functioning as described herein. Therefore, all related structural analogs which mimic the active structure of those formed by the compositions or proteins claimed herein are specifically included within the scope of the present invention.

Modified proteins are also within the contemplation of this patent application. These modifications may be deliberate, *e.g.*, modifications obtained through site-directed mutagenesis, or may be accidental, *e.g.*, as those obtained through mutation of the hosts.

Further, as is the case for all proteins, the precise chemical structure depends on a number of factors. As ionizable amino and carboxyl groups are present in the molecule, a particular protein may be obtained as an acidic or basic salt, or in neutral form. All such preparations which retain their activity when placed in suitable environmental conditions are included in the definition. Additionally, the primary amino acid sequence may be augmented by derivatization using sugar moieties (glycosylation) or by other supplementary molecules such as lipids, phosphate, acetyl groups and the like, more commonly by conjugation with saccharides. The primary amino acid structure may also aggregate to form complexes, most frequently dimers. Certain aspects of such augmentation are accomplished through post-translational processing systems of the producing host; other such modifications may be introduced *in vitro*. In any event, such modifications are included in the definition

so long as the activity of the protein is not destroyed. It is expected that such modifications may quantitatively or qualitatively affect the activity, either by enhancing or diminishing the activity of the protein in various assays.

Individual amino acid residues in the chain may also be modified by oxidation, reduction, or other derivatization, and the protein may be cleaved to obtain fragments which retain activity. Such alterations which do not destroy activity do not remove the protein sequence from the definition. The following discusses some of the modifications in further detail by way of example.

Thus, glycosylation variants are included within the scope of BDV. They include variants completely lacking in glycosylation (unglycosylated) and variants having at least one less glycosylated site than the native form (deglycosylated) as well as variants in which the glycosylation has been changed. Included are deglycosylated and unglycosylated amino acid sequence variants, deglycosylated and unglycosylated BDV and gp18 having the native, unmodified amino acid sequence of BDV and gp18, and other glycosylation variants, *e.g.* of p57. For example, substitutional or deletional mutagenesis is employed to eliminate the N- or O-linked glycosylation sites of BDV or gp18, *e.g.*, an asparagine residue is deleted or substituted for by another basic residue such as lysine or histidine. Alternatively, flanking residues making up the glycosylation site are substituted or deleted, even though the asparagine residues remain unchanged, in order to prevent glycosylation by eliminating the glycosylation recognition site.

Unglycosylated protein which has the amino acid sequence of the native protein is preferably produced in recombinant prokaryotic cell culture because prokaryotes are incapable of introducing glycosylation into polypeptides.

Glycosylation variants are produced by selecting appropriate host cells or by *in vitro* methods. Yeast, for example, introduce glycosylation which varies significantly from that of mammalian systems. Similarly, mammalian cells having a different species (*e.g.* hamster, murine, insect, porcine, bovine or ovine) or tissue origin (*e.g.* lung, liver, lymphoid, mesenchymal or epidermal) than the source of the BDV antigen are routinely screened for the ability to introduce variant glycosylation as characterized for example by elevated levels of mannose or variant ratios of mannose, fucose, sialic acid, and other sugars typically found in mammalian glycoproteins. *In vitro* processing of the proteins of the present invention typically is accomplished by enzymatic hydrolysis, *e.g.* endoglycosidase digestion.

Derivatization with bifunctional agents is useful for preparing intermolecular aggregates of BDV proteins and their derivatives with polypeptides as well as for cross-linking the protein and derivatives to a water insoluble support matrix or surface for use in the assay or affinity purification of its ligands. In addition, a study of intrachain cross-links will provide direct information on conformational structure. Commonly used cross-linking agents include sulfhydryl reagents, 1,1-bis(diazoacetyl)-2-phenylethane, glutaraldehyde, N-hydroxysuccinimide esters, for example esters with 4-azidosalicylic acid, homobifunctional imidoesters including disuccinimidyl esters such as 3,3'-dithiobis (succinimidyl-propionate), and bifunctional maleimides such as bis-N-maleimido-1,8-octane.

Certain post-translational derivatizations are the result of the action of recombinant host cells on the expressed polypeptide. Glutaminyl and asparaginyl residues are frequently post-translationally deamidated to the corresponding glutamyl and aspartyl residues.

Alternatively, these residues are deamidated under mildly acidic conditions. Either form of these residues falls within the scope of this invention.

Other post-translational modifications include hydroxylation of proline and lysine, phosphorylation of hydroxyl groups of seryl or threonyl residues, methylation of the α-amino groups of lysine, arginine, and histidine side chains {T.E. Creighton, *Proteins: Structure and Molecular Properties*, W.H. Freeman & Co., San Francisco, pp 79-86 (1983)}, acetylation of the N-terminal amine and, in some instances, amidation of the C-terminal carboxyl.

The claimed proteins are preferably produced using recombinant technologies. The nucleotide, *e.g.*, DNA or RNA, sequences which encode the desired polypeptides are amplified by use of *e.g.* the polymerase chain reaction in the case of DNA (hereinalso referred to as "PCR"), and reverse transcriptase-polymerase chain reaction (RT-PCR) in the case of RNA. Oligonucleotide sequences to be used as primers which can specifically bind to the ends of the regions of interest are synthesized. After the desired region of the gene has been amplified the desired sequence is incorporated into an expression vector which is transformed into a host cell. The nucleotide sequence is then expressed by the host cell to give the desired polypeptide which is harvested from the host cell. Plant, bacterial, yeast, insect, viral and mammalian expression systems may be used. Vectors which may be used in these expression systems may contain fragments of plant, bacterial, yeast, insect, viral, and/or mammalian origins.

Given the teachings contained herein, one skilled in the art can create the sequences disclosed herein, either by hand or with an automated apparatus. As examples of the current state of the art relating to polynucleotide synthesis, one is directed to Maniatis *et al., Molecular Cloning--A Laboratory Manual*, Cold Spring Harbor Laboratory (1984),

and Horvath *et al.*, *An Automated DNA Synthesizer Employing Deoxynucleoside 3'-Phosphoramidites*, Methods in Enzymology 154: 313-326, 1987.

Identification of Nucleotide Sequences, Cloning , and Expression of the Disclosed Protein

Alternatively, to obtain RNA encoding the proteins disclosed herein, one needs only to conduct hybridization screening with labelled BDV nucleotide sequence (usually, greater than about 20, and ordinarily about 50bp) in order to detect clones which contain homologous sequences in the cDNA libraries derived from cells or tissues of a particular animal, followed by analyzing the clones by restriction enzyme analysis and nucleic acid sequencing to identify full-length clones. The cell lines, cells and tissues are preferably from the nervous system, bone marrow, peripheral blood, mononuclear cells or brain of BDV infected animals. Examples of cells from the nervous system are: neurons, oligodendrocytes and astrocytes. The primers shown in Examples 1 to 4 and/or the methods shown therein may also be used.

If full length clones are not present in the library, then appropriate fragments are recovered from the various clones and ligated at restriction sites common to the fragments to assemble a full-length clone.

The techniques shown in this section are also useful for identifying and sequencing various isotypes and strains of BDV and BDV related viruses. The present invention discloses the nucleotide sequences of two strains of BDV; different strains of BDV may exist or arise due to mutation as in the case of human immunodeficiency virus (HIV) which constantly mutates and of which different strains are constantly being discovered. Thus, within the definition of BDV are other BDV isotypes and strains or viruses related to BDV ("BDV related viruses"). For example, the next section of the application describes diagnostic assays for BDV or related pathogenesis. The related

pathogenesis include: (1) diseases caused by BDV; (2) opportunistic or attendant diseases arising from BDV infection; and (3) diseases caused by BDV related viruses. The BDV related viruses would be nonsegmented, negative-stranded, neurotropic, post transcriptionally modified (spliced) viruses which share some homology with BDV nucleotide or amino acid sequences. Patients infected by the BDV related viruses would manifest clinical symptoms similar to BDV infected patients, or to that of neurologic or neuropsychiatric diseases.

Thus, DNA or RNA encoding various BDV isotypes and strains, and BDV related viruses, can be similarly obtained by probing libraries from cells and tissues, especially cells of the nervous system, of animals exhibiting clinical symptoms of BDV infection, neurologic or neuropsychiatric disease; or animals that have been purposely infected with BDV strains, isotypes or BDV related viruses, such as shown in Example 2. Once the DNA or RNA sequence of these strains, isotypes, or related viruses are known, primers based on the sequence may be used. The methods shown in Examples 1 and 2, and the primers shown therein may also be used to obtain the genomic nucleotide sequences.

In general, prokaryotes are used for cloning of DNA sequences in constructing the vectors useful in the invention. For example, *E. coli* K12 strain 294 (ATCC No. 31446) is particularly useful. Other microbial strains which may be used include *E. coli* B and *E. coli* X1776 (ATCC No. 31537). These examples are illustrative rather than limiting. Alternatively, *in vitro* methods of cloning, *e.g.* polymerase chain reaction, are suitable.

The proteins of this invention may be expressed directly in recombinant cell culture as an N-terminal methionyl analogue, or as a fusion with a polypeptide heterologous to the hybrid/portion, preferably a signal sequence or other polypeptide having a specific cleavage site at the N-terminus of the hybrid/portion. For

example, in constructing a prokaryotic secretory expression vector for portion/fragment of BDV protein, the native BDV signal is employed with hosts that recognize that signal. When the secretory leader is "recognized" by the host, the host signal peptidase is capable of cleaving a fusion of the leader polypeptide fused at its C-terminus to the desired mature BDV protein. For host prokaryotes that do not process the BDV signal, the signal is substituted by a prokaryotic signal selected, for example, from the group of the alkaline phosphatase, penicillinase, lpp or heat stable enterotoxin II leaders. For yeast secretion the BDV signal may be substituted by the yeast invertase, alpha factor or acid phosphatase leaders. In mammalian cell expression, the native signal is satisfactory for mammalian BDV, although other mammalian secretory protein signals are suitable, as are viral secretory leaders, for example the herpes simplex gD signal.

The proteins of the present invention may be expressed in any host cell, but preferably are synthesized in mammalian hosts. However, host cells from prokaryotes, fungi, yeast, insects and the like are also are used for expression. Exemplary prokaryotes are the strains suitable for cloning as well as *E. coli* W3110 (F-λ-A-prototrophic, ATTC No. 27325), other enterobacteriaceae such as *Serratia marcescans*, bacilli and various pseudomonads.

Expression hosts typically are transformed with DNA encoding the proteins of the present invention which has been ligated into an expression vector. Such vectors ordinarily carry a replication origin (although this is not necessary where chromosomal integration will occur). Expression vectors also include marker sequences which are capable of providing phenotypic selection in transformed cells, as will be discussed further below. For example, *E. coli* is typically transformed using pBR322,

a plasmid derived from an *E. coli* species {Bolivar, *et al.*, *Gene* 2:95 (1977)}. pBR322 contains genes for ampicillin and tetracycline resistance and thus provides easy means for identifying transformed cells, whether for purposes of cloning or expression. Expression vectors also optimally will contain sequences which are useful for the control of transcription and translation, *e.g.*, promoters and Shine-Dalgarno sequences (for prokaryotes) or promoters and enhancers (for mammalian cells). The promoters may be, but need not be, inducible; even powerful constitutive promoters such as the CMV promoter for mammalian hosts may produce BDV proteins without host cell toxicity. While it is conceivable that expression vectors need not contain any expression control, replicative sequences or selection genes, their absence may hamper the identification of transformants and the achievement of high level peptide expression.

Promoters suitable for use with prokaryotic hosts illustratively include the β-lactamase and lactose promoter systems {Chang *et al.*, *Nature* 275:615 (1978); and Goeddel *et al.*, *Nature* 281:544 (1979)}, alkaline phosphatase, the tryptophan (trp) promoter system (Goeddel, *Nucleic Acids Res.* 8:4057 (1980) and EPO Appln. Publ. No. 36,776) and hybrid promoters such as the tac promoter {H. de Boer *et al.*, *Proc. Natl. Acad. Sci. USA* 80:21-25 (1983)}. However, other functional bacterial promoters are suitable. Their nucleotide sequences are generally known, thereby enabling a skilled worker operably to ligate them to DNA encoding the proteins of the present invention {Siebenlist *et al.*, *Cell* 20:269 (1980)} using linkers or adaptors to supply any required restriction sites. Promoters for use in bacterial systems also will contain a Shine-Dalgarno (S.D.) sequence operably linked to the DNA encoding the proteins of the present invention

In addition to prokaryotes, eukaryotic microbes such as yeast or filamentous fungi are satisfactory.

Saccharomyces cerevisiae is the most commonly used eukaryotic microorganism, although a number of other strains are commonly available. The plasmid YRp7 is a satisfactory expression vector in yeast {Stinchcomb, *et al.*, *Nature* 282:39 (1979); Kingsman *et al.*, *Gene* 7:141 (1979); Tschemper *et al.*, *Gene* 10:157 (1980)}. This plasmid already contains the trp1 gene which provides a selection marker for a mutant strain of yeast lacking the ability to grow in tryptophan, for example ATCC no. 44076 or PEP4-1 {Jones, *Genetics* 85:12 (1977)}. The presence of the trp1 lesion as a characteristic of the yeast host cell genome then provides an effective environment for detecting transformation by growth in the absence of tryptophan. Alternatively, viral expression vectors such as retroviral vectors, baculoviral vectors and Semliki Forest viral vectors are used. The expression hosts of these vectors are known in the art.

Suitable promoting sequences for use with yeast hosts include the promoters for 3-phosphoglycerate kinase {Hitzeman *et al.*, *J. Biol. Chem.* 255:2073 (1980)} or other glycolytic enzymes {Hess *et al.*, *J. Adv. Enzyme Reg.* 7:149 (1968); and Holland, *Biochemistry* 17:4900 (1978)}, such as enolase, glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucos isomerase, and glucokinase.

Other yeast promoters, which are inducible promoters having the additional advantage of transcription controlled by growth conditions, are the promoter regions for alcohol dehydrogenase 2, isocytochrome C, acid phosphatase, degradative enzymes associated with nitrogen metabolism, metallothionein, glyceraldehyde-3-phosphate dehydragenase, and enzymes responsible for maltose and galactose utilization. Suitable vectors and promoters

for use in yeast expression are further described in R. Hitzeman *et al.*, European Patent Publication No. 73,657A.

Expression control sequences are known for eukaryotes. Virtually all eukaryotic genes have an AT-rich region located approximately 25 to 30 bases upstream from the site where transcription is initiated. Another sequence found 70 to 80 bases upstream from the start of transcription of many genes is a CXCAAT region where X may be any nucleotide. At the 3' end of most eukaryotic genes is an AATAAA sequence which may be the signal for addition of the poly A tail to the 3' end of the coding sequence. All of these sequences may be inserted into mammalian expression vectors.

Suitable promoters for controlling transcription from vectors in mammalian host cells are readily obtained from various sources, for example, the genomes of viruses such as polyoma virus, SV40, adenovirus, MMV (steroid inducible), retroviruses (*e.g.* the LTR of BDV), hepatitis-B virus and most preferably cytomegalovirus, or from heterologous mammalian promoters, *e.g.* the beta actin promoter. The early and late promoters of SV40 are conveniently obtained as an SV40 restriction fragment which also contains the SV40 viral origin of replication. {Fiers *et al., Nature* 273:113 (1978)}. The immediate early promoter of the human cytomegalovirus is conventionally obtained as a HindIII E restriction fragment. {Greenaway, P.J. *et al., Gene* 18:355-360 (1982)}.

Transcription of a DNA encoding the proteins of the present invention by higher eukaryotes is increased by inserting an enhancer sequence into the vector. Enhancers are cis-acting elements of DNA, usually about from 10-300bp, that act on a promoter to increase its transcription. Enhancers are relatively orientation and position independent having been found 5' {Laimins *et al.*, *Proc. Natl. Acad. Sci.*, 78:993 (1981)} and 3' {Lusky, M.L., *et al.*, *Mol. Cell Bio.* 3:1108 (1983)} to the transcription unit,

within an intron {Banerji, J.L. *et al.*, *Cell* 33:729 (1983)} as well as within the coding sequence itself {Osborne, T.F., *et al.*, *Mol. Cell Bio.* 4:1293 (1984)}. Many enhancer sequences are now known from mammalian genes (globin, elastase, albumin, α-fetoprotein and insulin). Typically, however, one will use an enhancer from a eukaryotic cell virus. Examples include the SV40 enhancer on the late side of the replication origin (bp 100-270), the cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers.

Expression vectors used in eukaryotic host cells (yeast, fungi, insect, plant, animal, human or nucleated cells from other multicellular organisms) will also contain sequences necessary for the termination of transcription which may affect mRNA expression. These regions are transcribed as polyadenylated segments in the untranslated portion of the mRNA. The 3' untranslated regions also include transcription termination sites.

Expression vectors may contain a selection gene, also termed a selectable marker. Examples of suitable selectable markers for mammalian cells are dihydrofolate reductase (DHFR), thymidine kinase (TK) or neomycin. When such selectable markers are successfully transferred into a mammalian host cell, the transformed mammalian host cell is able to survive if placed under selective pressure. There are two widely used distinct categories of selective regimes. The first category is based on a cell's metabolism and the use of a mutant cell line which lacks the ability to grow independent of a supplemented media. Two examples are CHO DHFR- cells and mouse LTK cells. These cells lack the ability to grow without the addition of such nutrients as thymidine or hypoxanthine. Because these cells lack certain genes necessary for a complete nucleotide synthesis pathway, they cannot survive unless the missing nucleotides are provided in a supplemented media. An alternative to supplementing the

media is to introduce an intact DHFR or TK gene into calls lacking the respective genes, thus altering their growth requirements. Individual cells which were not transformed with the DHFR or TK gene will not be capable of survival in non-supplemented media.

The second category of selective regimes is dominant selection which refers to a selection scheme used in any cell type and does not require the use of a mutant cell line. These schemes typically use a drug to arrest growth of a host cell. Those cells which are successfully transformed with a heterologous gene express a protein conferring drug resistance and thus survive the selection regimen. Examples of such dominant selection use the drugs neomycin {Southern *et al.*, *J. Molec. Appl. Genet.* 1:327 (1982)}, mycophenolic acid {Mulligan *et al.*, *Science* 209:1422 (1980)} or hygromycin {Sugden *et al.*, *Mol. Cell. Biol.* 5:410-413 (1985)}. The three examples given above employ bacterial genes under eukaryotic control to convey resistance to the appropriate drug G418 or neomycin (geneticin), xgpt (mycophenolic acid) or hygromycin, respectively.

"Amplification" refers to the increase or replication of an isolated region within a cell's chromosomal DNA. Amplification is achieved using a selection agent, *e.g.* methotrexate (MTX) which inactivates DHFR. Amplification or the making of successive copies of the DHFR gene results in greater amounts of DHFR being produced in the face of greater amounts of MTX. Amplification pressure is applied notwithstanding the presence of endogenous DHFR, by adding ever greater amounts of MTX to the media. Amplification of a desired gene can be achieved by cotransfecting a mammalian host cell with a plasmid having a DNA encoding a desired protein and the DHFR or amplification gene permitting cointegration. One ensures that the cell requires more DHFR, which requirement is met by replication of the selection gene, by selecting only for cells that can grow

in the presence of ever-greater MTX concentration. So long as the gene encoding a desired heterologous protein has cointegrated with the selection gene replication of this gene gives rise to replication of the gene encoding the desired protein. The result is that increased copies of the gene, *i.e.* an amplified gene, encoding the desired heterologous protein express more of the desired protein.

Suitable eukaryotic host cells for expressing the proteins include monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (293 or 293 cells subcloned for growth in suspension culture, {Graham, F.L. et al., *J. Gen Virol.* 36:59 (1977)}; baby hamster kidney cells (BHK, ATCC CCL 10); chinese hamster ovary-cells-DHFR {CHO, Urlaub and Chasin, *Proc. Natl. Acad. Sci., (USA)* 77:4216, (1980)}; mouse sertoli cells {TM4, Mather, J.P., *Biol. Reprod.* 23:243-251 (1980)}; monkey kidney cells (CV1 ATCC CCL 70); african green monkey kidney cells (VERO-76, ATCC CRL-1587); human cervical carcinoma cells (HELA, ATCC CCL 2); canine kidney cells (MDCK, ATCC CCL 34); buffalo rat liver cells (BRL 3A, ATCC CRL 1442); human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2, HB 8065); mouse mammary tumor (MMT 060562, ATCC CCL51); TRI cells {Mather, J.P., *et al., Annals N.Y. Acad. Sci.* 383:44-68 (1982)}; and C_6 glial cell (ATCC CCL 107).

Construction of suitable vectors containing the desired coding and control sequences employ standard ligation techniques. Isolated plasmids or DNA fragments are cleaved, tailored, and religated in the form desired to form the plasmids required.

For analysis to confirm correct sequences in plasmids constructed, the ligation mixtures are used to transform *E. coli* K12 strain 294 (ATCC 31446) and successful transformants selected by ampicillin or tetracycline resistance where appropriate. Plasmids from the transformants are prepared, analyzed by restriction and/or sequenced by the method of Messing *et al.*, *Nucleic Acids*

Res. 9:309 (1981) or by the method of Sanger *et al.*, *Proc. Natl. Acad. Sci., (USA)*, 74:5463 (1977).

Host cells are transformed with the expression vectors of this invention and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants or amplifying the genes encoding the desired sequences. The culture conditions, such as temperature, pH and the like, are those previously used with the host cell selected for expression, and will be apparent to the ordinarily skilled artisan.

The host cells referred to in this disclosure encompass cells in *in vitro* culture as well as cells which are within a host animal.

Diagnostic, Prognostic, and Monitoring Uses of BDV proteins and their derivatives

Another aspect of the present invention presents assays for detecting ligands, *e.g.*, in the biological samples of a test organism, which bind BDV protein(s) or derivatives thereof. These assays are useful as diagnostic tests for: (1) infection by BDV or related pathogenesis; and (2) neurologic and neuropsychiatric disease not due to BDV infection.

The preferred assays are immunoassays which detect antibodies to BDV proteins or its derivatives that are antigenic (herein referred to as "BDV antigen"). The test organism can be human or other animals, such as cats, fowls, ostriches, and horses. The biological samples may be biological fluids such as whole blood, serum, plasma, cerebral spinal fluid, or synovial fluid. Preferably, BDV proteins or its derivatives are used to detect the ligand by binding to it. Preferably, the ligand is an antibody directed to the polypeptides, and BDV antigens are used to detect the antibody. For example, the assay can be used to detect antibodies against BDV in biological fluids.

Alternatively, antibodies to BDV protein(s) or their derivatives can be used to screen for BDV proteins, *e.g.*, in the biological samples of a test organism. Similarly, the alternative detection of antibodies or antigen applies to each of the assay formats described below.

Thus, an example of the assay is an enzyme immunoassay. In an example of a direct assay, these polypeptides serve as antigens and are attached to a solid phase and then incubated with patient sera. Human serum or plasma is preferably diluted in a sample diluent before incubation. If antibodies to BDV are present in the sample they will form an antigen-antibody complex with the polypeptides and become affixed to the solid phase.

After the antigen-antibody complex has formed, unbound materials and reagents are removed by washing the solid phase and the antigen-antibody complex is reacted with a solution containing labelled antibodies directed against the first type of antibody. For example, the labelled antibody can be horseradish peroxidase-labeled goat antibody. This peroxidase labelled antibody then binds to the antigen-antibody complex already affixed to the solid phase. In a final reaction the horseradish peroxidase is contacted with o-phenylenediamine and hydrogen peroxide which results in a yellow-orange color. The intensity of the color is proportional to the amount of antibody which initially binds to the polypeptide affixed to the solid phase.

Another assay format provides for an antibody-capture assay in which anti-immunoglobulin antibody on the solid phase captures the patient's antibody, which is then reacted with the BDV antigen. The application of this format is similar to the serological assay of Lyme disease taught in Berardi et al., *J. Infect. Dis.* 158:754-760 (1988). If antibody to BDV is present, it captures the BDV antigen, and the bound BDV antigen is detected by means of labelled polyclonal or monoclonal antibodies

directed against the BDV antigen. The antibody-capture assay is particularly useful for and can increase the sensitivity of detection of IgM and IgG to BDV antigens. In an example of this assay, the fluid sample is first contacted with a solid support containing a bound antibody capable of binding the mu-chain of IgM or the gamma-chain of IgG antibodies. Specific antibody is detected by reacting this with the BDV antigens followed by non-human antibody to the BDV antigens. The non-human antibody is generally labelled for detection. It is believed that this antibody-capture immunoassay format will have increased sensitivity, especially for IgM. Alternatively, one can forego the non-human antibody and instead label the BDV antigens for direct detection.

Another assay format provides for an immunodot assay for identifying the presence of an antibody that is immunologically reactive with specific BDV antigens by contacting a sample with the BDV antigens bound to a solid support under conditions suitable for complexing the antibody with the BDV antigens and detecting the antibody-antigen complex by reacting the complex.

Suitable methods and reagents for detecting an antibody-antigen complex in an assay of the present invention are commercially available or known in the relevant art. For example, the detector antibodies or polypeptides may be labelled with enzymatic, radioisotopic, fluorescent, luminescent, or chemiluminescent label. These labels may be used in hapten-labelled antihapten detection systems according to known procedures, for example, a biotin-labelled antibiotin system may be used to detect an antibody-antigen complex.

In all of the assays, the sample is preferably diluted before contacting the BDV antigen absorbed on a solid support. Solid support materials may include cellulose materials, such as paper and nitrocellulose; natural and synthetic polymeric materials, such as

polyacrylamide, polystyrene, and cotton; porous gels such as silica gel, agarose, dextran and gelatin; and inorganic materials such as deactivated alumina, magnesium sulfate and glass. Suitable solid support materials may be used in assays in a variety of well known physical configurations, including microtiter wells, test tubes, beads, strips, membranes, and microparticles. A preferred solid support for a non-immunodot assay is a polystyrene microwell, polystyrene beads, or polystyrene microparticles. A preferred solid support for an immunodot assay is nitrocellulose or nylon membrane.

In particular, the invention presents an ELISA which is a rapid, sensitive, and inexpensive diagnostic test. The preferred ELISAs are based on recombinant BDV proteins recp40, recp23, and recp18. These assays are more sensitive and rapid than prior art methods employed for serologic diagnosis of infection, such as Western blot, indirect immunofluorescent test or immunoprecipitation.

Examples of the neurologic and neuropsychiatric diseases that can be diagnosed include diseases of the nervous system such as schizophrenia, depressive disorders (*e.g.*, bipolar depression), multiple sclerosis and AIDS-related encephalopathy. The finding is based on applicants' analysis of the art. Although the virus has not been recovered from human subjects, antibodies reactive with BDV proteins have been found in patients with bipolar depression, schizophrenia, or AIDS-related encephalopathy {Bode, L., et al., *Arch. Virol. Suppl.*, 7:159-167 (1993); Bode, L., et al., *Lancet*, ii:689 (1988) and Rott, R., et al., *Science* 228:755-756 (1985)}. BDV has a unique tropism for specific brain regions. Viral nucleic acids and disease-specific proteins have been found in highest concentrations in the hippocampus and limbic circuits, prefrontal and cingulate cortices, and brainstem nuclei {Carbone, K., et al., *J. Neuropathol. Exp.*

Neurol., 50:205-214 (1991); Ludwig, H., et al., *Prog. Med. Virol.* 35:107-151 (1988) and Solbrig, M. V., et al., abstr. 10, Abstr. 1992 Am. Acad. Neurol. Annu. Meet., (1992)}. Three BDV proteins, p40, p23 and gp18 (disclosed in Example 2 below) have been identified in infected cells and tissues {Ludwig, H., et al., *Prog. Med. Virol.* 35:107-151 (1988) and Thiedemann, N., et al., *J. Gen. Virol.*, 73:1057-1064 (1992)}. cDNAs for p40 {Lipkin, W. I., et al., *Proc. Natl. Acad. Sci. USA*, 87:4184-4188 (1990); McClure, M. A., et al., *J. Virol.*, 66:6572-6577 (1992) and Pyper, J. M., et al., *Virology*, 195:229-238 (1993)} and p23 {Lipkin, W. I., et al., *Proc. Natl. Acad. Sci. USA*, 87:4184-4188 (1990); Thierer, J., et al., *J. Gen. Virol.*, 73:413-416 (1992) and VandeWoude, S., et al., *Science*, 250:1276-1281 (1990)} have been isolated, and complementary sequences to open reading frames (ORFs) for these proteins have been mapped to the viral genome {Briese, T., et al., *Proc. Natl. Acad. Sci. USA* 91:4362-4366 (1994) which is incorporated into Example 1 of this application; and Cubitt, B., et al., *J. Virol.*, 68:1382-1996 (1994)}.

The assay can also be used to monitor the diseases by monitoring the titer of such ligands. The titer of the ligands, and the specific viral proteins that it is immunoreactive with, can also be prognosticative of the diseases.

Thus, an application of this invention may involve contacting the test subject's biological sample, such as serum, with a panel consisting of different immunogenic fragments of BDV protein or its derivatives. These proteins may be synthetic or native proteins, though recombinant proteins are preferred. Such a panel may consist of, for example, recp23, recp40, recp57, recpol and recp18. If the serum is immunoreactive with at least one of the fragments, it indicates that the test subject may either be suffering from (1) BDV or related

pathogenesis; or (2) neurologic and neuropsychiatric disease not due to BDV infection. Further, given a fixed amount of sample tested, the amount (*i.e.* percentage) of ligands immunoreactive with the BDV proteins may also be indicative of the severity of the disease and thus its prognosis. Generally, the higher the percentage of ligands that are immunoreactive, the more severe the disease and the poorer the prognosis. Thus, the assay may also be used to monitor the progress of the disease. In particular, if the test subject is undergoing treatment for the disease, the assay may be used to monitor the efficacy of the drug and treatment regimen. Such monitoring may involve assaying for the ligand titer and/or the specific BDV immunogenic epitopes which the ligand binds to.

Hybridization Diagnostic Assays

Oligonucleotides ("probes") that are unique, or relatively unique to BDV in a test sample, are useful for diagnosing BDV infections. Nucleotide hybridization assay may be used, whereby nucleic acids from a patient's biological sample are contacted to the primers or BDV restriction fragments under hybridization condition, and the hybridization products are detected. This method could be used to detect viral genomic RNA or mRNA. Conventional Western or Northern Blot analysis, RT-PCR or PCR and ligase chain reaction (LCR) may be used as the basis of the assay, these techniques are known to those skilled in the art. PCR and LCR techniques are widely available in the art. For example, the basic PCR techniques are described in United States Patent Nos. 4,683,202; 4,683,195; 4,800,159; and 4,965,188. The basic LCR techniques are described in EPA-320,308; EPA-439,182; EPA-336,731; WO 89/09835; WO 89/12696, and WO 90/01069.

Since the present invention presents the full nucleotide sequence of the genomic BDV nucleotide

sequence, these probes can be identified by comparing this sequence with the sequences of other organisms which may contaminate a test sample. Such comparison can be conducted as described in Example 1 below or using methods known in the art. The probes preferably contain at least 10 contiguous nucleotides or at least 30 contiguous nucleotides with at least 60% homology along the length of the BDV nucleotide sequence being compared. Examples of such probes and methods for conducting the PCR for detection are as described in Examples 1 and 2.

Assay Kits

The present invention also encompasses immunoassay kits containing BDV antigen(s), preferably each antigen per container, in a concentration suitable for use in immunoassay. In the kits, the BDV antigens may be bound to a solid support and where needed, the kits may include sample preparation reagents, wash reagents, detection reagents and signal producing reagents.

Also included are assay kits for nucleotide hybridization assays which include probes which are specific for BDV or its derivatives. The kits may also include sample preparation reagents, wash reagents, detection reagents and signal producing reagents.

Therapeutic Uses of Antibodies Directed to BDV proteins and Their Derivatives

Another aspect of the invention presents methods, using antibodies directed to BDV proteins or derivatives, for treating: (1) BDV infection or related pathogenesis; and (2) neurologic and neuropsychiatric disease not due to BDV infection. Examples of such antibodies are those specific to gp18 and p57. The antibodies may be administered using methods known in the art. Preferably, this involves passive administration of these antibodies, such as those described in Example 4.

Peptides Useful For Diagnostics and Therapeutics

Another aspect of the invention presents peptides *e.g.* the truncated fragments and peptides disclosed in "EXAMPLE 5", below, containing at least one BDV immunoepitope. These peptides can be used in diagnostic assays to detect the presence of a patient's antibodies agaisnt BDV. Thus, the peptides are useful for the assays described in the section: "Diagnostic, Prognostic, and Monitoring Uses of BDV proteins and their derivatives". For example, as shown in Example 3 below, recp40, recp23, and recp18 have proved useful for detecting BDV infections. Thus, the epitopes of these recombinant proteins can be mapped, and smaller peptides containing these epitopes and routinely tested for their immunoreactivity with antibodies to BDV, *e.g. using the ELISA method shown in Example 3.*

The above peptides can also be used to raise antibodies that may serve as therapeutics against BDV infections such as shown in Example 4 and as described in the section: "Therapeutic Uses of Antibodies Directed to BDV proteins and Their Derivatives". Examples of methods for synthesizing peptide fragments are described in Stuart and Young in "Solid Phase Peptide Synthesis", 2nd ed., Pierce Chemical Co. (1984). It is contemplated that antibodies which precipitate BDV viral particles would be useful for therapeutic uses. In particular, these antibodies are raised against proteins, and their fragments, expressed on the surface of BDV. It is further contemplated that antibodies against gp18, p57 and their fragments, especially antibodies that precipitate BDV viral proteins would be useful for treating or preventing the disease (1) BDV infection or related pathogenesis; and (2) neurologic and neuropsychiatric disease not due to BDV infection.

Thus, fragments of BDV proteins, in particular gp18 and p57 and their fragments, can be made starting from either end of their C-termini and NH_2-termini. For

example, these fragments can be tested according to the ELISA method shown in Example 3 against, *e.g.* sera from horses, rats, or human patients infected with BDV. The fragments that react with the sera would be useful for detecting the disease and would be useful for raising therapeutic antibodies to treat the disease. Since different animals may react to different epitopes of BDV proteins, one may even tailor the screening test by using the serum from the same species of animal for which one seeks to develop an assay or therapeutic. For example, if one is seeking a diagnostic test or therapeutic for humans, the sera tested will be preferably that from human patients. Included in this invention are other methods, known in the art, for identifying the immunoreactive epitopes of a protein and raising antibodies thereto. Further, since antibodies which are immunoreactive with BDV protein may also be found in the sera of patients with neurologic and neuropsychiatric disease not necessarily due to BDV infection, the above peptides and antibodies raised thereto may also find usefulness in diagnosing, monitoring and treating these patients. Additionally, these peptides may be identified by their immunoreactivity with sera from patients suffering from neurologic and neuropsychiatric disease not due to BDV infection. Thus, as described in this application, the disease, patient sera to be tested, the diagnostic, monitoring and therapeutic uses are not limited to BDV, and include (1) BDV infection or related pathogenesis; and (2) neurologic and neuropsychiatric disease not due to BDV infection. Further, one can screen for therapeutic ligands or chemicals which bind these peptides. These therapeutic chemicals then may be tested for their therapeutic effect against the above diseases. Other ligands or chemicals which bind the therapeutic ligands or chemicals can be tested for their ability to bind patients' antisera or antibodies and are thus useful as diagnostics for the diseases.

Preferably, the above peptides and antibodies are also respectively tested for their crossreactivity with antibodies raised by and proteins from organisms unrelated to the above diseases but commonly found in the test sample (*e.g.* patient's biological sample). Peptides and antibodies that are highly non-specific are preferably not used. To obtain peptides of high specificity, one may also compare the amino acid sequence of BDV protein with that of known contaminating proteins in the test sample. The fragments that are unique, or relatively so, to BDV are then chosen for further screening as described above, *e.g.* for immunoreactivity with patient's test sample. These comparison can also be done on the nucleotide sequence level.

Method for Producing Antibodies to BDV and its Derivatives

Besides whole immunoglobulins, antibodies herein include antigen binding fragments of the immunoglobulins. Examples of these fragments are Fab, F(ab')2 and Fv. Such fragments can be produced by known methods. Unless otherwise indicated, antibodies herein also include: polyclonal and monoclonal antibodies, monospecific antibodies, and antisera which includes monospecific antisera.

Antibodies to BDV proteins and their derivatives can be produced using standard procedures known in the art. For example, antibodies can be produced by inoculating a host animal such as a rabbit, rat, goat, mouse, etc., with BDV proteins and their derivatives. Before inoculation, the polypeptides or fragments may be first conjugated with keyhole limpet hemocyanin (KLH) or bovine serum albumin (BSA). After an appropriate time period for the animal to produce antibodies to the polypeptides or fragments, the anti-serum of the animal is collected and the polyclonal antibodies separated from the anti-serum using techniques known in the art. Monoclonal

antibodies can be produced by the method described in Kohler and Milstein (*Nature*, 256:495-497, 1975) by immortalizing spleen cells from an animal inoculated with the polypeptides or fragments thereof. The immortalization of the spleen cell is usually conducted by fusing the cell with an immortal cell line, for example, a myeloma cell line, of the same or different species as the inoculated animal. The immortalized fused cell can then be cloned and the cell screened for production of the desired antibody.

The antibodies may also be recombinant monoclonal antibodies produced according to the methods disclosed in Reading, United States Patent No. 4,474,893, or Cabilly et al., United States Patent No. 4,816,567. The antibodies may also be chemically constructed according to the method disclosed in Segel et al., United States Patent No. 4,676,980.

While the invention is demonstrated using mouse monoclonal antibodies and rat monospecific antisera, the invention is not so limited. In fact, human antibodies may be used and may prove to be preferable. The latter is especially so if the antibodies are used as therapeutics for humans, as there would be less immunorejection from the human patients receiving these antibodies. Such antibodies can be obtained by using human hybridomas {Cote et al., Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, p. 77 (1985)}. In fact, according to the invention, techniques developed for the production of chimeric antibodies {Morrison et al., Proc. Natl. Acad. Sci., 81:6851 (1984); Neuberger et al., Nature, 312: 604 (1984); Takeda et al., Nature, 314: 452 (1985)} by splicing the genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity (such as ability to activate human complement and mediate antibody-dependent cell-mediated

cytotoxicity) can be used; such antibodies are within the scope of this invention.

VACCINE

By providing the nucleotide and amino acid sequences of the BDV genome and BDV proteins, respectively, this application enables the production of recombinant BDV {*e.g.* using the technique shown in Schnell, M. J., EMBO J., 13: 4195-4203 (1994)} which can then be attenuated, *e.g.* by mutagenesis, heat or formaldehyde treatment, to be used as vaccine against (1) BDV infection or related pathogenesis; and (2) neurologic and neuropsychiatric disease not due to BDV infection. BDV sequences, their mutagenized sequences or fragments thereof, may be administered, *e.g.* by direct injection, or incorporated into a vector and administered *e.g.* by direct injection, into patients. Examples of the fragments are the truncated fragments and peptides disclosed in "EXAMPLE 5", below. The injections may be by means of a gene gun. gp18, p57, pol, and proteins produced by the mutagenized or fragmented sequences may also serve as vaccines. Proteinaceous vaccines may be delivered orally, intravenously, intraperitoneally, or intramuscularly. The vaccine may also be contained in a physiologically compatible solution.

BDV Viral Vector Based Delivery System

Another aspect of the invention presents: (A) a BDV-mediated gene transfer for the incorporation and expression of eukaryotic or prokaryotic foreign genes into another eukaryotic or prokaryotic host; and (B) an *in vitro* BDV-mediated delivery of gene(s) or chemical(s) to a target cell.

In Method A, one or more desired genes are inserted into the BDV viral vector. The desired gene transfer can be achieved through *in vitro* transfection of a cell or cell

line by the resulting BDV viral vector. The transfected cell or cell line thus expresses the gene(s) of interest and the expression product(s) are harvested. Alternatively, the transfected cell or cell line is later transplanted into a host, *e.g.* an animal such as a human, in need of the gene product(s). In this case, the gene(s) is expressed *in vivo*. The generation of infectious non-segmented, neurotropic, negative-stranded RNA virus entirely from cloned cDNA, has been described in the case of rabies virus {Schnell, M. J., et al., EMBO J., 13(18): 4195-4203 (1994)}. The insertion of foreign gene(s) into the BDV viral vector is based on prior art teachings for other viral vectors, which may include insertion of promoters or regulators to control expression of the foreign gene(s). The transfection and gene therapy is similarly based on prior art teaching for viral vectors. Such teachings abound, see *e.g.*, U.S. Patent No. 5,219,740 to Miller et al., Jun 15, 1993; U.S. Patent No. 5,256,553 to Overell, Oct. 26, 1993; and WO 91/12329, assigned to Board of Regents, the University of Texas System, international publication date, Aug. 22, 1991.

Method B utilizes the unique tropism of BDV for specific regions and cells of the nervous systems, *e.g.* neural cells. Thus, BDV vector can be used for *in vivo* delivery of chemicals or desired genes to these regions. For example, infectious recombinant BDV containing the gene of interest can be used to infect the specific target cells of BDV in a host animal. The host can be a human suffering from deficiency, lack of, or a malfunctioning of the gene product. The general gene therapy methods can be based on prior art teaching *e.g.* the references cited for Method A, such as WO 91/12329. In the case of BDV viral vectors, these genes can be those responsible for the survival, proliferation, and proper functioning of the nervous system. For example, in neurodegenerative diseases, the cells in the patients'

nervous system suffer premature death, and these cells are not regenerated, eventually causing the patients to die. The inserted gene(s) may supplement or replace the dysfuntional gene(s) in these patients to provide gene product(s) necessary for continued survival and proliferation of these cells. Examples of the inserted genes include genes coding for: neurotransmitters, cytokines, growth factors, receptors for the foregoing, enzymes for activation of therapeutic drugs administered to the patients.

Alternatively, the viral vector may contain a nucleotide sequence coding for a toxin. These vectors would infect the host's cells *in vivo*, express the toxin and kill the infected cells. The targeted cells are preferably neoplastic cells, or cells infected by or harboring pathogenic organisms. The vector is preferably further designed to selectively target these cells over normal cells. One means to target the desired cells is by localized injection of the recombinant virus, containing the desired gene, near the target of interest. However, for BDV based gene therapy, the vector or recombinant virus may be delivered peripherally, *i.e.* into subcutaneous tissue, peripheral nerve, or intramuscularly. The neurotropism of the recombinant virus allows it to migrate towards cells of the nervous system to transfect or infect them.

The BDV viral vector is an especially good vehicle for gene therapy and *in vivo* chemical delivery. It has several advantages over the viral vectors known in the art, the most common of which are retroviral vectors. Retroviral vectors require replication of its host cells for transfection. Therefore, retroviral vectors can only be used with dividing/mitotic cells. In contrast, BDV vectors are autonomous, self-replicating vectors and thus can transfect both dividing and non-dividing cells. Thus, BDV is particularly effective for transfecting

nerve cells that normally do not divide and for which BDV is tropic.

Further, BDV does not have a latent stage in its lifecycle, after transfecting a host cell. It thus will continue to express the desired gene once it has transfected a cell. This is unlike some viral vectors currently used in the art, such as the herpes viral vector that may enter a latent stage after transfection and thus not express the desired gene product in the transfected cell. BDV is also unique in that it is a slow growing virus and is not lytic. Thus, chances of the virus lysing and killing the host cells are nonexistent.

As a further safeguard, the BDV viral vectors may be made infective but replication-defective, rendering them useful vectors which are unable to produce infective virus, following introduction into a cell. For initiation of productive infection of BDV, a nucleocapsid containing BDV genomic RNA is required, from which primary transcription of mRNAs and ensuing autonomous and regulated expression of all BDV proteins occurs. Thus, to render the viral vector replication-defective, one may mutate the nucleocapsid protein produced by recombinant virus to prevent encapsidation of newly synthesized genomic RNA. Additionally, the host cell should preferably be devoid of infectious helper virus which may assist in replication of the BDV. Further, unlike retroviruses and herpes viruses, BDV does not cause disease in and of itself. The deleterious effect of BDV infection is actually caused by the host's immune-mediated rejection of BDV and BDV antigen expressed on infected cells. The rejection involves cellular immune response which activates the host's effector lymphocytes which then kill the transfected cells. Antibodies appear not to be as important in the host's immune response. Thus, one means to avoid Borna disease is to interfere with, avoid, or suppress the host's ability to recognize

or mount an immune response to BDV infected cells. For example, immune response in the host is triggered when T lymphocytes recognize a complex of major histocompatibility complex (MHC) and foreign antigen (in this case, BDV proteins) expressed on the host cell's surface. Thus, to reduce the host's immune response, one may choose to interfere with or prevent the expression of MHC on the transfected cells. This may be achieved by inserting, into the BDV viral vector, a nucleotide sequence which codes for a mRNA (*i.e.* an antisense mRNA) which would bind the mRNA coding for the component of MHC ("$mRNA_{MHC}$") and prevent the translation and expression of MHC in the transfected cell. Absent MHC, the BDV antigens will not be presented on the host cell surface to trigger immune-mediated rejection in the host. Alternatively, other methods known in the art may be used to avoid the immune rejection of BDV transfected cells.

EXAMPLE 1

Cloning and Sequencing of Genomic RNA from Borna Disease Virus (BDV) Particles

The studies in this example and Example 2, except with regard to p57, are also described in Briese, T., et al., *Proc. Natl. Acad. Sci., USA*, 91:4362-4366 (1994) and Kliche, S., et al., *J. Virol.*, 68: 6918-6923 (1994), respectively, both of which are hereby incorporated by reference in their entirety. In this example, the BDV genome was cloned to reveal antisense information for five open reading frames (ORFs). From 5' to 3' on the antigenome, the ORFs are p40, p23, gp18, p57 and pol. Proteins p40, p23 and gp18 have been identified in infected cells and tissues: p40 and p23 are expressed at high levels *in vitro* and *in vivo* and are found in the nucleus and cytoplasm of infected cells {Bause-Niedrig, I., M. et al., *Vet. Immunol. Immunopathol.*, 31:361-369 (1992)}. gp 18 is a membrane-

associated glycoprotein that is expressed at lower levels. gp18 was characterized in Example 2 below.

Messenger RNAs {Kliche, S., et al., *J. Virol.*, 68: 6918-6923 (1994); Lipkin, W. I., et al., *Proc. Natl. Acad. Sci. USA*, 87:4184-4188 (1990); McClure, M. A., et al., *J. Virol.*, 66:6572-6577 (1992); Pyper, J. M., et al., *Virology*, 195:229-238 (1993); Thibault, K. J., M.S. thesis; University of California, Irvine (1992); Thierer, J., et al., *J. Gen. Virol.*, 73:413-416 (1992) and VandeWoude, S., et al., *Science*, 250:1278-1281 (1990)} and proteins {Bause-Niedrig, I., et al., *Vet. Immunol. Immunopathol.*, 31:361-369 (1992); Haas, B., et al., *J. Gen. Virol.*, 67:235-241 (1986); Ludwig, H., et al., *Progr. Med. Virol.*, 35:107-151 (1988); Schädler, R., et al., *J. Gen. Virol.*, 66:2479-2484 (1985) and Thiedemann, N., et al., *J. Gen. Virol.*, 73:1057-1064 (1992)} corresponding to three of these ORFs, p40, p23 and gp18, have been found in infected cells and tissues in a 5'-3' expression gradient (p40 > p23 > gp18) {Briese, T., et al., *Proc. Natl. Acad. Sci. USA*: 91:4362-4366 (1994); Cubitt, B., et al., *J. Virol.*, 68:1382-1396 (1994); and Richt, J. A., et al., *J. Gen. Virol.*, 72:2251-2255 (1991)}.

Though Cubitt, B., et al., *J. Virol.*, 68:1382-1396 (1994) purported to have sequenced the BDV genome, their paper contains numerous errors. The errors included (1) failure to recognize deletions in subgenomic RNAs due to splicing; (2) misplacement of ORFs leading to the prediction of a 40kD protein instead of a 57kD protein and failure to detect ORF overlap of p57 with gp18 and pol; and (3) selection of incorrect motifs for initiation of transcription. These mistakes were implicitly acknowledged in a subsequent paper, de la Torre, J. C., *J. Virol.*, 68:7669-7675 (1994). Figure 1 of the latter paper incorporated the correct genomic organization and transcription map described in Example 1 of this application. A later minireview which compares the

sequence differences between the above Cubitt, et al.'s genomic sequence and the sequence described in Example 1 below concludes that the differences seem most likely due to cloning and/or sequencing errors (of Cubitt et al.'s) rather than natural differences between the nucleotide sequences of different strains. Schneeman, A., et al., to be published in *Virology*, 209 (1995); a co-author of the paper is Dr. Robert A. Lamb, the editor-in-chief of *Virology* and a Howard Hughes Medical Institute Investigator. Dr. Lamb was not a collaborator in the work described in Example 1 below..

In this Example, the 8,910 nucleotide BDV viral genome was cloned and sequenced using RNA from BDV particles. The viral genome has complementary 3' and 5' termini and contains antisense information for five open reading frames. Homology to Filo-, Paramyxo- and Rhabdoviridae is found in both cistronic and extracistronic regions. Northern analysis indicates that the virus transcribes mono- and polycistronic RNAs and uses termination/polyadenylation signals reminiscent of those observed in other negative-strand RNA viruses. BDV is likely to represent a previously unrecognized genus, bornaviruses, or family, Bornaviridae, within the order Mononegavirales.

MATERIALS AND METHODS

BDV cDNA Library Preparation and Screening.

Genomic RNA template for library construction was obtained from an oligodendrocyte cell line (Oligo/TL) acutely infected with BDV Strain V {Briese, T., et al., *Proc. Natl. Acad. Sci. USA* 89:11486-11489 (1992)}. For the first genomic library, RNA from one viral particle preparation was polyadenylated with poly(A) polymerase (GibcoBRL, Life Technologies, Inc., Grand Island, New York) to facilitate cloning from the 3' terminus by oligo d(T) primed cDNA synthesis. Libraries were prepared in pSPORT using the SuperScript Plasmid system (GibcoBRL, Life

Technologies, Inc., Grand Island, New York). The first library was screened using pAB5 and pAF4 radiolabeled restriction fragments {Lipkin, W. I., et al., *Proc. Natl. Acad. Sci. USA* 87:4184-4188 (1990)}. Subsequent libraries were screened using radiolabeled restriction fragments from locations progressively 5' on the genomic RNA. 5'-terminal sequence from each library was used to design an oligonucleotide primer for construction of the next library.DNA sequencing and sequence analysis. Plasmid DNA was sequenced on both strands by the dideoxynucleotide chain termination method {Sanger, F., et al., *Proc. Natl. Acad. Sci. USA* 74:5463-5467 (1977)} using bacteriophage T7 DNA polymerase (Sequenase version 2.0; United States Biochemical, Cleveland, Ohio). Five to ten independent clones from each library were sequenced with overlap so that each region of the genomic RNA was covered by at least two clones. Four libraries were analyzed yielding ~8.9 kb of continuous sequence. Nucleic acid sequence was analyzed using the Sequence Analysis Software Package (Genetics Computer, Inc., Madison, Wisconsin). Database searches for related sequences and multiple sequence alignments were performed using FastA and Pileup.

Sequence Determination at the 3' and 5' Termini of BDV Genomic RNA. Genomic RNA from one viral particle preparation (1-2 x 10^8 cells) was treated with tobacco acid pyrophosphatase (Epicentre Technologies, Madison, Wisconsin) and circularized with T4 RNA ligase (New England Biolabs, Inc., Beverly, Massachusetts) {Mandl, C. W., et al., *BioTechniques* 10:484-486 (1991)}. The ligated RNA was reverse transcribed with Superscript II (Gibco BRL, Life Technologies, Inc., Grand Island, New York) using primer 5'-GCCTCCCCTTAGCGACACCCTGTA (SEQ ID NO: 11), complementary to a region 465 nucleotides (nt) from the 5' terminus of the BDV genome. A 2 μl aliquot of the reverse transcription reaction was used to amplify the ligated region by the polymerase chain reaction (PCR)

using Stoffel fragment (Perkin-Elmer Cetus, Norwalk, Connecticut). Primers used in the first round of PCR were 5'-GCCTCCCCTTAGCGACACCCTGTA (SEQ ID NO: 11) and 5'-GAAACATATCGCGCCGTGCA (SEQ ID NO: 12), located 241 nt from the 3' terminus of the BDV genome. Amplified products were subjected to a second round of PCR using a nested set of primers: 5'-TACGTTGGAGTTGTTAGGAAGC (SEQ ID NO: 13), 251 nt from the 5' terminus, and 5'-GAGCTTAGGGAGGCTCGCTG (SEQ ID NO: 14), 120 nt from the 3' terminus. PCR products were cloned {Schneider, P. A., et al., *J. Virol.* 68:63-68 (1994)} and sequence across the 5'/3' junction was determined from five independent isolates.

Northern hybridization. Poly(A)$^+$ enriched RNA extracted from acutely infected rat brain using FastTrack (Invitrogen Corp., San Diego, California) was size-fractionated on 0.22 M formaldehyde/1.0% agarose gels {Tsang, S. S., et al., *BioTechniques* 14:380-381 (1993)}, transferred to Zeta-Probe GT nylon membranes (Bio-Rad Laboratories, Richmond, California) and hybridized with random-primed 32P-labeled restriction fragments {Feinberg, A. P., et al., *Anal. Biochem.* 132:6-13 (1983)} representing ORFs across the BDV genome (FIG. 6 b). RNA transfer, hybridization and washing were performed following the manufacturer's protocol (Bio-Rad Laboratories, Richmond, California).

RESULTS

The following figures present some of the results:

FIG. 3. (a) Organization of the BDV genome. Hatched boxes represent coding sequence complementary to ORFs for identified proteins, p40, p23, gp18, or putative proteins, p57, p180. (p180 is also referred to as pol.) Overlap is indicated by cross-hatched areas. Length of coding sequence corresponding to ORFs in nucleotides is indicated in brackets. Underlined italic numbers indicate length of sequence from stop codon complement to

last templated uridine of termination/polyadenylylation signal (black boxes). Italics with arrow indicate number of nucleotides in intervening sequence between p40 polyadenylylation signal and p23 coding sequence and between p23 polyadenylylation signal and gp18 coding sequence, respectively. Italics with dashed arrow indicate number of noncoding nucleotides at termini of the genome. (b) Coding potential of genome. Genomic sequence was translated in all six possible reading frames (3'-5' negative sense; 5'-3' positive sense) by using FRAMES (Genetics Computer Group). ORFs are indicated by bars and hatched boxes.

FIG. 4. Alignment of the p180 (pol) ORF and negative-strand RNA virus L-polymerase amino acid sequences with PILEUP. Solid lines indicate conserved L-polymerase motifs (a, A, B, C, D). BDV sequence is indicated with double arrowheads. Rhabdoviridae: RaV, rabies virus; VSV, vesicular stomatitis virus; SYN, sonchus yellow net virus. Paramyxoviridae: MeV, measles virus; SeV, Sendai virus; NDV, Newcastle disease virus; RSV, respiratory syncytial virus. Filoviridae: MaV, Marburg virus. Numbers indicate amino acid range shown. Uppercase letters in viral sequence lines indicate residues conserved in more than six sequences. Uppercase letters in consensus line (Con) indicate presence of identical or conserved amino acids in BDV. Agreement of BDV sequence with either rhabdo- or paramyxoviruses is indicated by * or x, respectively. +, Nonconserved glycine residue in BDV.

FIG. 5. Sequence analysis of BDV genomic termini. (a) Similarity of 3'-terminal BDV sequence to leader regions of Rhabdoviridae (RaV), VSV), Paramyxoviridae (MeV, SeV, NDV, RSV), and Filoviridae (MaV). Abbreviations are as in FIG. 2. EboV, Ebola virus. Sequences are aligned by using arbitrary gap insertion to optimize nucleotide matching. (b) Comparison of complementarity at 3' and 5' termini of BDV genomic RNA

with that of four other nonsegmented, negative-strand RNA viruses. The 3′ and 5′ terminal sequences for each virus are shown in viral RNA (3′-5′, negative sense) orientation. Underlined sequence refers to transcriptional start of first gene or end of the L-polymerase gene (also referred to as "pol gene"), respectively (predicted for BDV). The end of the L-polymerase gene of RaV is located outside the region shown.

FIG. 6. Map of BDV subgenomic RNAs relative to the viral antigenome. (a) Northern hybridization analysis of rat brain poly(A)$^+$ RNA. Each lane was hybridized with a probe representing a major BDV ORF as indicated by the letters A-E (see b). Results of hybridization with probes C* and E* were identical to results of hybridization with probes C and E, respectively (data not shown). Numbers at left indicate size of RNA markers in kilobases. Numbers at right indicate estimated size of major transcripts. (b) Position of viral transcripts with respect to antigenome as determined by Northern hybridization and sequence analysis. Dashed lines indicate regions in the 1.5-kb RNA and the 6.1-kb RNA that contain a deletion. The boundaries of the deletions are not known. Relative positions of probes used for Northern hybridization are shown. On the ORF map, potential start codons are indicated with upward lines; ◇, start codons predicted to be functional; x, potential start codon present in strain V that is absent in strain He/80 (see text). Potential termination sites are indicated with downward lines. Use of T2 and T3 has been confirmed {McClure, M. *et al., J. Virol.*, 66:6572-6577; Thierer, J. *et al., J. Gen. Virol.*, 73:413-416}; use of T5 and T7 is consistent with hybridization results. Termination at t1, t4 and t6 has not been observed (see a). (c) Alignment of the seven potential termination sites of BDV. Location of sites is indicated in the ORF map. Stop codons are underlined. Lowercase letters indicate

termination/polyadenylylation consensus sequence. No termination/polyadenylylation site was found at or near the end of the gp18 ORF.

Sequencing of Genomic BDV RNA.

Beginning from the 3' terminus, a series of four overlapping cDNA libraries was constructed using BDV particle RNA {Briese, T., et al., *Proc. Natl. Acad. Sci. USA* 89:11486-11489 (1992)} as template. Previous studies have shown that the genomic RNA is not polyadenylated {de la Torre, J., et al., *Virology* 179:853-856 (1990)}. Thus, to construct the first library, genomic RNA was polyadenylated *in vitro* in order to facilitate oligo d(T)-primed cDNA synthesis. For the subsequent three libraries, genome-complementary oligonucleotide primers were designed based on 5' terminal sequence determined in the previous round of cloning. Each region of the genome was sequenced using a minimum of two independent clones. To determine the sequences at the termini, genomic RNA was circularized and sequenced across the junction using five independent clones.

The 8,910 nt BDV genome contained antisense information for five major ORFs flanked by 53 nt of noncoding sequence at the 3' terminus and 91 nt of noncoding sequence at the 5' terminus (FIG. 3). In 3'-5' order, the first two ORFs encoded two previously described viral proteins, p40 {McClure, M. A., et al., *J. Virol.* 66:6572-6577 (1992)} and p23 {Thierer, J., et al., *J. Gen. Virol.* 73:413-416 (1992)}. The third, fourth and fifth ORF had coding capacities of 16 kDa (gp18), 57 kDa (p57) and 190kDa (p180), respectively (FIG. 3a). Note: p180 is now known as "pol". Predicted amino acid sequence for the 16 kDa ORF correlated with microsequence data for an 18 kDa BDV glycoprotein (see the section below for gp18 glycoprotein), originally described as the Borna disease-associated 14.5 kDa protein {Schädler, R.,

et al., *J. Gen. Virol.* 66:2479-2484 (1985)}. The first three ORFs showed no overlap and were in frame with the fifth ORF (FIG. 3b). The 57 kDa ORF was in a +1/-2 frame relative to the other four ORFs and overlapped the adjacent ORF for gp18 by 28 amino acids and ORF p180 by 34 amino acids. All ORFs were located on the (+) strand, complementary to the genomic RNA. ORF analysis of the genomic (-) strand showed only three small ORF's, each with a coding capacity of less than 16 kDa (FIG. 3b).

Homology analysis of coding sequence.

Predicted amino acid sequence for the identified ORFs was used to examine databases for similarity to other proteins. Previous analysis of the ORF encoding p40 had revealed distant sequence similarity to L-proteins of Paramyxoviridae and Rhabdoviridae {McClure, M. A., et al., *J. Virol.* 66:6572-6577 (1992)}. FastA analysis of translated sequence from ORFs p23, gp18 and p57 showed no apparent similarity to other viral sequences; however, ORF p180 sequence consistently retrieved L-polymerases of Paramyxo- and Rhabdoviridae. Alignment of ORF p180 (pol) sequence with sequence of RNA-dependent RNA polymerases of negative-strand RNA viruses showed conservation of both sequence and linear order of regions homologous among these proteins. Extensive conservation was found in the four characteristic motifs for L-polymerases of negative-strand RNA viruses (A-D in FIG. 4) {Poch, O., et al., *EMBO J.* 8:3867-3874 (1989) and Poch, O., et al., *J. Gen. Virol.* 71:1153-1162 (1990)}. With the exception of the glycine residue in motif B (position 322 of the alignment), conservation was found for the individual amino acid residues postulated to participate in polymerase function {Poch, O., et al., *EMBO J.* 8:3867-3874 (1989)}. Conservation was also found for a motif (a in FIG. 4) proposed to participate in template recognition

{Poch, O., et al., *J. Gen. Virol.* 71:1153-1162 (1990) and Barik, S., et al., *Virology* 175:332-337 (1990)}. The GCG/pileup alignment placed ORF p180 sequence between polymerases of Paramyxo- and Rhabdoviridae. This intermediate position is reflected by the presence of conserved amino acids which are in agreement with either the rhabdo- or the paramyxovirus sequences (* or x, respectively; FIG. 4). The distance between conserved motifs a and A was found to be short in BDV as it is in rhabdoviruses, whereas this region is highly variable in length and sequence among paramyxoviruses {Poch, O., et al., *J. Gen. Virol.* 71:1153-1162 (1990)}. The GCG/pileup generated dendrogram, obtained using complete ORF p180 and L-protein sequences, indicated that the putative BDV polymerase was more closely related to L-polymerases of Rhabdoviridae than Paramyxoviridae.

Analysis of Noncoding Sequence at the Genomic Termini.

3' terminal genomic sequence had a high A/U content of 60.5 % with an A to U ratio of ~1:2, similar to 3' leader sequences of other negative-strand RNA viruses. At the extreme 3' end, filo-, paramyxo- and rhabdoviruses have a common G/U rich region (FIG. 5a). In BDV, as in respiratory syncitial virus, rabies virus and filoviruses, this region was not located at the 3' extremity. Comparison of the 3' and 5' termini of BDV genomic RNA revealed complementarity similar to that found in other negative-strand RNA viruses {Keene, J. D., et al., *J. Virol.* 32:167-174 (1979) and Tordo, N., et al., *Virology* 165:565-576 (1988)} (FIG. 5b). Alignment of the genomic termini allowed formation of a terminal panhandle, with the first three nucleotides unpaired. The subsequent complementary area of 6 nucleotides (positions 4-9 and 8907-8902) could be extended by one gap insertion between position 8901/8,902 resulting in an

additional 10 nt stretch of complementarity with a single mismatch (positions 18 and 8994; FIG. 5b).

Identification of Potential Termination/ Polyadenylation Sites.

Sequence preceding the poly(A) tracts of two cloned BDV mRNAs (UA_5) {McClure, M. A., et al., *J. Virol.* 66:6572-6577 (1992) and Thierer, J., et al., *J. Gen. Virol.* 73:413-416 (1992)} was used to analyze genomic sequence for homologous sites that could serve as potential termination/ polyadenylation signals. Seven sites were found (FIG. 6 c). Northern hybridization experiments supported use of four of these sites (T2, T3, T5 and T7) and allowed identification of a termination/polyadenylation signal consensus sequence ($CMNMYYMNWA_6$), where M is A or C, Y is C or U, and W is A or U. Only one of the three remaining sites (t6) matched the consensus sequence (FIG. 6c).

Northern Hybridization Analysis.

Restriction fragments representing the five ORFs were used as probes for hybridization to poly(A)$^+$ enriched RNA isolated from acutely infected rat brain by FastTrack (FIG. 6a and b). Because this procedure does not entirely eliminate poly(A)$^-$ RNAs, small levels of BDV genome-size RNA can usually be detected in these preparations. To allow determination of the relative abundance of RNAs detected by each probe, exposure times were normalized to the signal of the 8.9-kb RNA. Consistent with the 3′ to 5′ transcriptional gradient found for other negative-strand RNA viruses, of the eight subgenomic RNAs identified, those detected by the 3′-most probes (genomic orientation), A and B, were more abundant than those detected by the more 5′ probes (FIG. 6a and b).

Mapping of the eight transcripts to the genome by Northern hybridization indicated use of only three sites

for transcriptional initiation and four sites for termination. Probes C* and E* were used to distinguish between termination at T5 or t6 (FIG. 6b). The patterns of hybridization with probes C* and E* were identical to those obtained with probes C and E, respectively indicating termination at T5 (data not shown). Probes corresponding to p40 (A) and p23 (B) detected monocistronic RNAs of 1.2 kb and 0.75 kb, respectively (FIG. 6). Probes A and B also detected a 1.9 kb RNA consistent with failure of transcriptional termination at the p40 termination site {Pyper, J. M., et al., *Virology* 195:229-238 (1993)}. Transcriptional readthrough was also found for polycistronic transcripts of 3.5, 2.8 kb and 7.1 kb. The 3.5 kb RNA detected by probes B, C, D and C*, is likely to initiate at or near the beginning of ORF p23 and terminate at T5. The 2.8 kb RNA detected by probes C, D and C*, is likely to initiate at or near the beginning of ORF gp18 and terminate at T5. The 7.1 kb detected by probes C, D, C*, E* and E, is likely to initiate at or near the beginning of ORF gp18 and to continue through T5 until it terminates at T7. Probes C and C* both hybridized to a 1.5 kb RNA and a 6.1 kb RNA. Interestingly, neither the 1.5 kb RNA nor the 6.1 kb RNAs was detected by probe D, located between C and C* on the viral genome. These findings are consistent with posttranscriptional modification resulting in a 1-1.3 kb deletion (FIG. 6).

DISCUSSION

The order Mononegavirales, which incorporates the families Filoviridae, Paramyxoviridae and Rhabdoviridae, has distinct characteristics that include: (1) a nonsegmented negative sense RNA genome, (2) linear genome organization in the order 3′ untranslated region /core protein genes /envelope protein genes /polymerase gene /untranslated 5′ region, (3) a virion associated RNA-dependent RNA polymerase, (4) a helical nucleocapsid

that serves as template for replication and transcription, (5) transcription of 5-10 discrete, unprocessed mRNAs by sequential interrupted synthesis from a single promoter and (6) replication by synthesis of a positive sense antigenome {Pringle, C. R., et al., *Arch. Virol.* 117:137-140 (1991)}. The genomes of rhabdo-, paramyxo- and filoviruses range in size from 11 to 20 kb. The BDV genome has been estimated to be between 8.5 {Lipkin, W. I., et al., *Proc. Natl. Acad. Sci. USA* 87:4184-4188 (1990) and de la Torre, J., et al., *Virology* 179:853-856 (1990)} and 10.5 kb {VandeWoude, S., et al., *Science* 250:1276-1281 (1990) and Richt, J., et al., *J. Gen. Virol.* 72:2251-2255 (1991)} in length. Our data confirm that the BDV genome, at only 8910 nt, is smaller than those of other negative-strand RNA viruses. Several features suggest that BDV is a member of the order Mononegavirales: organization of ORFs on the genome, extensive sequence similarities of the largest BDV ORF to L-polymerases of rhabdo-, paramyxo- and filoviruses, homology of 3' noncoding sequence to leader sequences of Mononegavirales and complementarity of BDV genomic termini.

In 5' to 3' antigenomic orientation, the first ORF contains 1110 nt. Due to a more favorable translation initiation context {Kozak, M., *Nucleic Acids Res.* 15:8125-8148 (1987)}, it is likely that the second AUG codon, 39 nt inside the ORF, is used to express a 357 aa protein of 39.5 kDa (p40) {Pyper, J. M., et al., *Virology* 195:229-238 (1993)}. 26 nt downstream of the stop codon is a polyadenylation signal {McClure, M. A., et al., *J. Virol.* 66:6572-6577 (1992)} (T2, FIG. 6 b and c). The second ORF starts 79 nt from the p40 polyadenylation site. It has a length of 603 nt coding for a 201 aa protein of 22.5 kDa (p23). The stop codon of ORF p23 is part of the polyadenylation signal {Thierer, J., et al., *J. Gen. Virol.* 73:413-416 (1992)} (T3, FIG. 6b and c). Analysis of the

intergenic region between ORFs p40 and p23 has shown that this sequence is less conserved among different BDV isolates than coding sequences for p40 and p23 {Schneider, P. A., et al., *J. Virol.* 68:63-68 (1994)}. Therefore, expression of a small ORF in this region (x, FIG. 3 b); {VandeWoude, S., et al., *Science* 250:1276-1281 (1990) and Pyper, J. M., et al., *Virology* 195:229-238 (1993)} that overlaps with ORF p23 seems unlikely {Schneider, P. A., et al., *J. Virol.* 68:63-68 (1994)}. Ten nt downstream of the p23 polyadenylation signal is the third ORF, 426 nt in length, that codes for a 142 aa (16.2 kDa) protein. Due to glycosylation, the protein expressed from this ORF has a Mr of ~18 kDa (gp18).

No polyadenylation signal similar to those identified for p40 and p23 mRNAs {McClure, M. A., et al., *J. Virol.* 66:6572-6577 (1992) and Thierer, J., et al., *J. Gen. Virol.* 73:413-416 (1992)} was found near the end of the gp18 ORF (FIG. 6b and c). Instead, the following ORF overlaps with the end of the gp18 ORF by 28 aa. It has a total size of 1,509 nt that could code for a 503 aa protein of 56.7 kDa (p57). The ORF has two AUG codons in the overlap with gp18. A third AUG located outside the overlap is 451 nt from the beginning of the ORF. Which, if any, of these AUGs is used is unknown as no protein has been identified. A potential polyadenylation site is located 28 nt downstream of the p57 ORF (t4). However, Northern hybridization results suggest that this site is a weak or nonfunctional signal, because no major transcript(s) were found to stop at this position (FIG. 6).

The fifth ORF encompasses more than half the length of the genome. A potential polyadenylation site (T7), similar to that seen at the end of ORFs p40 and p23, is found 33 nt from the stop codon of p180 (pol) ORF (FIG. 6b and c). Deletions identified by Northern hybridization analysis suggested that viral mRNAs might

undergo post-transcriptional modification by RNA splicing. This hypothesis was subsequently confirmed by applicants {Schneider, P.A. *et al., J. Virol.*, 68:5007-5012 (1994); Schneemann, A. *et al. J. Virol.*, 68:6514-6522 (1994), hereby incorporated in their entirety.} RNA splicing extends the pol ORF by 459 nucleotides allowing prediction of a protein of 190kDa. {Schneider, P. *et al., J. Virol.*, 68:5007-5012 (1994)}. Although functional studies of BDV proteins have not yet been done, the organization of the viral genome together with the limited biochemical data available suggest possible roles for individual proteins in the virus life cycle. Four lines of evidence suggest that p40 is likely to be a structural protein: (1) like nucleocapsid proteins (N) of rhabdo- and paramyxoviruses {Banerjee, A. K., et al., *Pharmacol, Ther.* 51:47-70 (1991)} (except pneumoviruses {Collins, P. L., *The Paramyxoviruses*, ed. Kingsbury, D. W. (Plenum, New York), pp. 103-162 (1991)}), p40 is found in the most 3' position on the genome; (2) p40 is similar in size to N proteins; (3) both p40 {Pyper, J. M., et al., *Virologyy* 195:229-238 (1993) and Ludwig, H., et al., *Prog. Med. Virol.* 35:107-151 (1988)} and N proteins {Banerjee, A. K., et al., *Pharmacol, Ther.* 51:47-70 (1991)} are abundant in infected cells and particles; (4) neither N proteins {Banerjee, A. K., et al., *Pharmacol, Ther.* 51:47-70 (1991)} nor p40 {Thiedemann, H., et al., *J. Gen. Virol.* 73:1057-1064 (1992)} are phosphorylated or glycosylated. p23, a phosphorylated protein {Thiedemann, H., et al., *J. Gen. Virol.* 73:1057-1064 (1992)}, is in the next position on the genome. ORF p23 corresponds in position to genes coding for phosphoproteins in Paramyxoviridae (P) and Rhabdoviridae (NS) {Banerjee, A. K., et al., *Pharmacol, Ther.* 51:47-70 (1991)}. This suggests that p23 might serve a similar role in the BDV system. In support of this hypothesis, GCG analysis showed that the protein has a

high Ser/Thr content (16%), is charged (pI 4.8) and contains a N-terminal cluster of acidic amino acids compatible with structural features of P/NS proteins {Banerjee, A. K., et al., *Pharmacol, Ther.* 51:47-70 (1991)}. In previously described Mononegavirales, the next gene codes for matrix protein (M) {Banerjee, A. K., et al., *Pharmacol, Ther.* 51:47-70 (1991)}. gp18 occupies this position on the BDV genome. Though small for a matrix protein, gp18 has a predicted pI ,10, that is close to the basic pI of M proteins, ~9, and its membrane-association would be compatible with a matrix protein function. For p57, computer analysis predicted similarities to glycoproteins of negative-strand RNA viruses: potential glycosylation sites as well as N-terminal and C-terminal hydrophobic "anchor" domains (data not shown). The largest ORF (pol) is located most 5' on the genome. Its size, 5' position and conservation of motifs considered critical to L-polymerase activity, suggest that this ORF is likely to code for the BDV polymerase (FIG. 6).

Analysis of Northern hybridization experiments in conjunction with genomic sequence data has allowed construction of a tentative transcription map (FIG. 6). While it has not been possible to identify signals for initiation of transcription by using consensus sequences of other negative-strand RNA viruses, we have identified consensus sequence for termination/polyadenylation in BDV using known ends of p40 and p23 mRNAs {McClure, M. A., et al., *J. Virol.* 66:6572-6577 (1992) and Thierer, J., et al., *J. Gen. Virol.* 73:413-416 (1992)} (FIG. 6c). These sequences appear to function as weak termination signals. Unlike other negative-strand RNA viruses, BDV shows a high frequency of readthrough transcripts. Organization and sequence similarities to Filo-, Paramyxo- and Rhabdoviridae suggest that BDV is a member of the order Mononegavirales. Dependent on the parameters and regions selected for homology analysis, BDV can be represented as

being more closely related to filo-, paramyxo- or rhabdoviruses. Overlap of coding sequence, high frequency of polycistronic readthrough transcripts and posttranscriptional modification are properties of the BDV system not found in other members of the order Mononegavirales. These features could serve as independent mechanisms for modulation of gene expression to achieve the persistent, non-cytopathic infection that is a cardinal characteristic of this neurotropic virus.

EXAMPLE 2

BDV Glycoprotein gp18

Using methods for isolation of the 14.5-kDa protein {Schädler, R., et al., *J. Gen. Virol.*, 66:2479-2484 (1985)}, we have purified a glycoprotein from BDV-infected rat brain that is encoded by a 429-nucleotide (nt) ORF located 3' to ORF p23 on the viral antigenome. The protein is predicted to be 16.2 kDa; glycosylation results in a 1- to 2-kDa increase in molecular weight. This glycoprotein, gp18, is the first glycoprotein to be identified in the BDV system. Lectin binding and endoglycosidase sensitivity assays suggest that gp18 is an unusual N-linked glycoprotein.

MATERIALS AND METHODS

Infection of animals and cultured cells.

Animals and cells were infected with BDV strain He/80 {Herzog, S., et al., *Med. Microbiol. Immunol.*, 168:153-158 (1980) and Schneider, P. et al., *Virol.* 68:63-68 (1994)}. Newborn Lewis rats were infected by intracranial injection with 1.5×10^4 focus-forming units of BDV. Three weeks after infection, animals were sacrificed and brains were removed for isolation of BDV particles {Carbone, K., et al., *J. Virol.*, 61:3431-3440 (1987)} or gp18. C6 cells and MDCK cells were persistently infected with BDV as described previously {Carbone, K. M., *J. Virol.*, 67:1453-1460 (1993) and Herzog,

S., et al., *Med. Microbiol. Immunol.*, 168:153-158 (1980)}. Monolayers of rabbit fetal glial cells were acutely infected by adding BDV at 1.0 focus-forming unit per cell to the culture medium (Dulbecco modified Eagle medium, 5% fetal calf serum; Gibco BRL, Grand Island, New York).

Protein purification and microsequencing.

Protein was purified from infected cells and tissues by detergent-salt extraction by the method of Schädler et al. {Schädler, R., et al., *J. Gen. Virol.*, 66:2479-2484 (1985)}. For microsequencing, protein was cleaved with 10% cyanogen bromide in 75% formic acid (Sigma Chemical Co., St. Louis, Missouri). Peptide fragments were separated by reverse-phase high-performance liquid chromatography (RP-HPLC) on a Vydac C-18 column, using a trifluoroacetic acidacetonitrile gradient. Sequence determinations were performed by automated Edman degradation on a Hewlett-Packard model G1000A protein sequencer.

Antibodies.

Antibodies to purified gp18 were produced in 3-month-old BALB/c mice. Animals were injected subcutaneously with 5 μg of protein in Freund's complete adjuvant and boosted 3 weeks later with a subcutaneous injection of 3 μg of protein in Freund's incomplete adjuvant. For 6 weeks thereafter, at 2-week intervals, animals received intraperitoneal injections of 5 μg of protein in phosphate-buffered saline (PBS) with 5 μg of lipopolysaccharide (*Salmonella typhimurium*; Difco, Detroit, Michigan) (three injections). Blood was drawn every 2 weeks during weeks 7 through 28 for measurement of serum antibody titer to purified protein by Western blotting (immunoblotting). Antisera collected at week 28 were used for virus neutralization studies. Rabbit antisera to recombinant BDV p40 and p23 were used as controls (see Example 3, below).

Cloning and sequencing of CDNA encoding gp18.

gp18-specific oligonucleotides were used to amplify full-length coding sequence for gp18 from two BDV-infected adult rat brain cDNA libraries {Lipkin, W. I., et al., *Proc. Natl. Acad. Sci. USA*, 87:4184-4188 (1990) and McClure, M. A., et al., *J. Virol.* 66:6572-6577 (1992)} as well as total cellular RNA {Chirgwin, J. J., et al., *Biochemistry*, 18:5294-5299 (1979)} and poly(A)$^+$ RNA {Aviv, H., et al., *Proc. Natl. Acad. Sci. USA*, 69:1408-1412 (1972)} extracted from infected rat brain. Reverse transcription (RT) was performed with an oligo(dT) primer and Superscript II (Gibco BRL, Life Technologies, Inc., Grand Island, New York). PCR was carried out with Ampli-Taq Stoffel fragment according to standard protocols (Perkin-Elmer, Norwalk, Connecticut) with the following primer pair: 5'-terminal *Xho*I-gp18 sense oligonucleotide (*Xho*I-gp18-S1), TCCTCGAGATGAATTCAAAACATTCCTATC (nt 1892 to 1914; *Xho*I restriction site indicated by underlining) (SEQ ID NO: 15); and 3'-terminal gp18 antisense oligonucleotide (gp18-AS1), CTAAGGCCCTGAAGATCGAAT (nt 2301 to 2321) (SEQ ID NO: 16). Products were purified by agarose gel electrophoresis using a USBioclean purification kit (U.S. Biochemical, Cleveland, Ohio) and cloned into Bluescript SKII+ (Stratagene, San Diego, California) prepared with 3' T overhangs {Marchuk, D., et al., *Nucleic Acid Res.*, 19:1154 (1990)}. A minimum of three independent clones from each template source was sequenced on both strands by the dideoxynucleotide chain termination method using bacteriophage T7 DNA polymerase (Sequenase; U.S. Biochemical, Cleveland, Ohio). The plasmid resulting from amplification of neonatally infected rat brain RNA was named pBDV-gp18.

***In vitro* transcription, translation, and cotranslational processing.**

Plasmid clones pBDV-gp18 and pBDV-23 {Thibault, K. J., M.S. thesis, University of California, Irvine (1992)} linearized with *Eco*RI were used as templates for *in vitro* synthesis of capped RNA transcripts. Transcription products or *Saccharomyces cerevisiae* α-factor mRNA (control for glycosylation) were translated *in vitro* by using nuclease-treated rabbit reticulocyte lysates (Promega Corp., Madison, Wisconsin) in the presence of [^{35}S]methionine (Amersham Corporation, Arlington Heights, Illinois). Cotranslational processing was assessed by *in vitro* translation using reticulocyte lysates supplemented with canine microsomal membranes (Promega, Madison, Wisconsin). Transcription, translation, and cotranslational processing studies were performed according to the manufacturer's protocols. Translation products were immunoprecipitated with mouse anti-gp18 serum and then size fractionated by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) (13% gel) {Laemmli, U. K., et al., *J. Mol. Biol.*, 80:575-581 (1973)} for autoradiographic analysis. Methods for immunoprecipitation and autoradiography have been described elsewhere {Lipkin, W. I., et al., *Proc. Natl. Acad. Sci. USA*, 87:4184-4188 (1990)}.

Protein gel electrophoresis and immunoblotting.

Proteins were size fractionated by SDS-PAGE (12% gel) and then transferred to Immobilon-N membranes (Millipore Corp., Bedford, Massachusetts). Primary antisera for immunoblotting were from rats chronically infected with BDV (day 100 after intracranial infection) or mice immunized with purified gp18. The secondary antibody was alkaline phosphatase-conjugated goat antimouse immunoglobulin G (Sigma Chemical Co., St.

Louis, Missouri); the substrate was Western Blue (Promega Corp., Madison, Wisconsin).

Carbohydrate analysis.

Purified protein was size fractionated by SDS-PAGE (13% gel) and then either silver stained for detection of protein or carbohydrate {Tsai, C. M., et al., *Anal. Biochem.*, 119:115-119 (1982)} or transferred to Immobilon-N membranes (Millipore, Bedford, Massachusetts) for lectin staining. The carbohydrate composition of immobilized protein was determined by using a DIG Glycan Differentiation Kit (Boehringer Mannheim, Indianapolis, Indiana) and peroxidase-labeled *Bandeiraea simplicifolia* agglutinins I and II (BS-I and BS-II; Sigma Chemical Co., St. Louis, Missouri). The substrate for peroxidase was 4-chloro-1-naphthol (Pierce Chemical Company, Rockford, Illinois). Glycosidase digests of native and denatured protein (incubated for 5 minutes at 100°C in 0.01% SDS) were performed according to the manufacturer's protocols, using the following endoglycosidases: endoglycosidase F and
N-glycosidase F; *O*-glycosidase; *N*-glycosidase F; endoglycosidase F, *N*-glycosidase free; endoglycosidase H; and endo-β-galactosidase (Boehringer Mannheim).

RESULTS

The following figures present some of the results:

FIG. 7. Sequence of ORF gp18. The diagram shows the location of ORF gp18 on the viral antigenome (5'-3') relative to ORFs p40 and p23 (boxes). ORF gp18 sequences were from Oligo/TL cells infected with BDV strain V (SV) and rat brain infected with BDV He/80 (RB). Peptide sequences (P#1, P#2, and P#3) were obtained by microsequencing of purified protein from He/80-infected rat brain. Periods indicate identical nucleotide or amino acid sequences. Variable amino acid residues (large asterisk) and stop codons (small asterisks) are

indicated. Underlining indicates potential glycosylation sites.

FIG. 8. Glycan determination of gp18. gp18 isolated from infected rat brain was size fractionated by SDS-PAGE (12% gel) then transferred to an Immobilon-N membrane for lectin staining (see Materials and Methods). Lanes: 0, protein detection by mouse anti-gp18 serum; 1, ConA; 2, wheat germ agglutinin; 3, *D. stramonium* agglutinin; 4, BS-I; 5, BS-II; 6, *G. nivalis* agglutinin; 7, *S. nigra* agglutinin; 8, *M. amrensis* agglutinin; 9, peanut agglutinin. Positions of molecular weight markers are shown in kilodaltons at the right.

FIG. 9. gp18 is sensitive to endoglycosidases. gp18 isolated from infected rat brain was treated with either buffer alone or endoglycosidase. Protein was size fractionated by SDS-PAGE (13% gel) and detected by silver staining. Lanes: 1, buffer; 2, endoglycosidase F plus *N*-glycosidase F; 3, endoglycosidase F (*N*-glycosidase free); 4, endo-β-galactosidase. Positions of molecular weight markers are shown in kilodaltons at the right.

FIG. 10. *In vitro* transcription, translation, and cotranslational processing of gp18. RNA transcripts were synthesized from pBDV-23 (a nonglycosylated BDV protein control) or pBDV-gp18 and translated *in vitro* by using rabbit reticulocyte lysates in either the absence or presence of canine microsomal membranes. [^{35}S]methionine-labeled translation products were immunoprecipitated with antisera to p23 or gp18 and protein A-Sepharose and then size fractionated by SDS-PAGE (13% gel) for autoradiography (A) or transferred to Immobilon-N membranes for ConA lectin staining (B). Translated gp18 in lane 5 of panel A and lane 3 of panel B was incubated with endoglycosidase F plus *N*-glycosidase F prior to SDS-PAGE. (A) Lanes: 1, pBDV-23 RNA; 2, pBDV-23 RNA plus microsomal membranes; 3, pBDV-gp18 RNA; 4, pBDV-gp18 RNA plus microsomal membranes; 5, pBDV-gp18 RNA plus

microsomal membranes, incubated with endoglycosidases. The long arrow indicates the position of glycosylated protein (lanes 3 and 4); the short arrow indicates the position of protein after treatment with endoglycosidase F plus *N*-glycosidase F (lane 5). The asterisk indicates nonspecific background signal (lane 5). Positions of molecular weight markers are shown in kilodaltons at the right. (B) Lanes: 1, pBDV-gp18 RNA; 2, pBDV-gp18 RNA plus microsomal membranes; 3, pBDV-gp18 RNA plus microsomal membranes, incubated with endoglycosidases.

Isolation of gp18.

Protein was isolated from neonatally infected rat brain, acutely infected rabbit fetal glial cells (two passages), persistently infected C6 cells, and persistently infected MDCK cells, using the method of Schädler et al. {Schädler, R., et al., *J. Gen. Virol.*, 66:2479-2484 (1985)}. The purity of the protein was confirmed by silver staining of the protein after SDS-PAGE (data not shown). The quantity of protein was estimated in silver-stained gels by using lysozyme standards. Typical yields were 5 μg of protein from one neonatally infected rat brain and 2 μg of protein from 10^8 infected cultured cells. Protein from neonatally infected rat brain was used for microsequencing, carbohydrate analysis, and immunization of mice.

Protein and nucleic acid sequence analysis.

Direct microsequencing of gp18 was not possible because of a blocked amino terminus; thus, to allow analysis, the protein was cleaved with cyanogen bromide. Sequencing of the cleavage mixture indicated the presence of three N termini. From the mixture, two peptides (peptides 1 and 3; FIG. 7) were isolated by RP-HPLC and sequenced individually, allowing inference of a third sequence (peptide 2; FIG. 7) by subtraction. Peptide sequences were used as probes to search ORFs located on

the BDV antigenome. The peptide sequences obtained from the purified gp18 mapped to a 429-nt ORF (ORF gp18) on the viral antigenome that predicts a 142-amino-acid protein with a molecular weight of 16,244 (FIG. 7).

Genomic sequence corresponding to the gp18 ORF was used to design probes and primers for identifying mRNA encoding gp18. In each of two cDNA libraries prepared from BDV-infected adult rat brain poly(A)$^+$ RNA {Lipkin, W. I., et al., *Proc. Natl. Acad. Sci. USA*, 87:4184-4188 (1990) and McClure, M. A., et al., *J. Virol.*, 66:6572-6577 (1992)}, 100,000 recombinants were screened by hybridization with a 271-bp *Hinc*II-*Hinf*I restriction fragment from pTB-BDV 5.82 (nt 2062 to 2333 in the viral genome) {Briese, T., et al., *Proc. Natl. Acad. Sci. USA* 91:4362-4366 (1994)}. These libraries were also screened by PCR using the 5'-terminal *Xho*I-gp18 sense primer (nt 1892 to 1914) and oligo(dT). Total cellular and poly(A)$^+$ RNAs extracted from persistently infected C6 cells, BDV-infected adult rat brain, or 3-week-old neonatally infected rat brain (the peak time point for *in vivo* expression of gp18) were subjected to RT-PCR using oligo(dT) in combination with the 5'-terminal *Xho*I-gp18 sense primer. No gp18-specific transcript corresponding to the size of ORF gp18 was obtained in these experiments. In contrast, use of the 5'-terminal *Xho*I-gp18 sense primer in combination with a 3'-terminal gp18 antisense primer (nt 2301 to 2321) allowed amplification of gp18 sequences from any of these sources by RT-PCR. In spite of variability at the nucleic acid level, the predicted amino acid sequence obtained from the different sources was the same as for strain V genomic sequence, with the exception of a single exchange in position 108 (E→D) (FIG. 7).

Characterization of gp18 as a glycoprotein.

Purified gp18 was size fractionated by SDS-PAGE. Modified silver staining revealed the presence of

carbohydrate; thus, fractionated protein was blotted onto Immobilon-N membranes to determine the presence of individual saccharides through lectin binding studies. Binding was observed with *Cancanavalia ensiformis* agglutinin (ConA), wheat germ agglutinin, *Datura stramonium* agglutinin, BS-I, and BS-II but not with *Galanthus nivalis* agglutinin, *Sambucus nigra* agglutinin, *Maackia amurensis* agglutinin, and peanut agglutinin (FIG. 8). This staining pattern was consistent with the presence of *N*-acetylglucosamine, *N*-acetylgalactosamine, mannose, and galactose. In addition, native and denatured proteins were digested with specific endoglycosidases, size fractionated by SDS-PAGE, and then stained to assess molecular weight shift and presence or absence of carbohydrate. Treatment with *O*-glycosidase or endoglycosidase H had no effect (data not shown). In contrast, treatment with endoglycosidase F and
N-glycosidase F resulted in a loss of 1 to 2 kDa (FIG. 9) and abrogation of lectin staining with ConA (data not shown). Treatment with endoglycosidase F (*N*-glycosidase free) or endo-β-galactosidase also resulted in a loss of 1 to 2 kDa (FIG. 9).

***In vitro* transcription, translation, and processing of gp18.**

With linearized pBDV-gp18 used as a template, gp18 RNA was transcribed and translated *in vitro* in either the presence or absence of canine microsomal membranes. The gp18 RNA directed translation of two proteins of 16 and 18 kDa that were recognized by monospecific murine antiserum to purified gp18. Translation in the presence of microsomal membranes led to an increase in the relative proportion of the 18-kDa protein. Treatment with endoglycosidase F resulted in loss of the 18-kDa protein species (FIG. 10A). Glycosylation of the 18-kDa species was also shown by lectin binding studies performed after translation products were size

fractionated by SDS-PAGE and transferred to membranes. The 18-kDa protein was recognized by ConA, whereas the 16-kDa protein did not bind ConA (FIG. 10B). Modification of translated protein by the microsomal membranes was specific for gp18. Translation of RNA encoding BDV p23, which encodes a potential N-glycosylation site (amino acids 53 to 55), included as a negative control for *in vitro* glycosylation, was not influenced by the presence of microsomal membranes (FIG. 10A).

DISCUSSION

We have isolated and partially characterized a BDV glycoprotein with unusual properties. This protein, previously reported as 14.5 kDa {Schädler, R., et al., *J. Gen. Virol.*, 66:2479-2484 (1985)}, is 16.2 kDa prior to carbohydrate modification and ~18 kDa after glycosylation. Though no classical sites for N linkage (N-x-S/T {Marshall, R. D., *Annu. Rev. Biochem*, 41:673-702 (1972)}) are found in the gp18 sequence, the protein is readily modified *in vitro* in the presence of a microsomal membrane system capable of N glycosylation {Gahmberg, C. G., et al., p. 281-297, *In* S. Fleischer and B. Fleischer (ed.), *Biomembranes*, Academic Press, New York (1983) and Lau, J. T. Y., et al., *J. Biol. Chem.*, 258:15255-15260 (1983)}. In addition, gp18 is sensitive to *N*-glycosidase F, an enzyme which cleaves between asparagine and *N*-acetylglucosamine {Plummer, T. H., et al., *J. Biol. Chem.*, 259:10700-10704 (1984) and Tarentino, A. L., et al., *Biochem.*, 24:4665-4671 (1985)}. These findings indicate that gp18 is N glycosylated at a nonclassical site. One potential site is N-I-Y (amino acids 74 to 76). The presence of a hydroxyl amino acid (T or S) or cysteine in position +2 (N-x-T/S or C) has been proposed as essential for hydrogen bond donor function in N glycosylation

{Bause, E., et al., *Biochem. J.*, 195:639-644 (1981)}. It is possible that tyrosine (Y), another hydroxyl amino acid in position +2, could serve as a hydrogen bond donor in gp18. A second potential site for N glycosylation is L-N-S-L-S (amino acids 87 to 91), which is similar to S-N-S-G-phosphorylated S, the site for N glycosylation in a glycopeptide from hen yolk phosvitin {Shainkin, R., et al., *J. Biol. Chem.*, 246:2278-2284 (1971)}.

gp18 is sensitive to endoglycosidase F, an enzyme that cleaves after the N-linked *N*-acetylglucosamine in high mannose-, biantennary hybrid-, and biantennary complex-type oligosaccharides {Tarentino, A. L., et al., *Biochem.*, 24:4665-4671 (1985) and Tarentino, A. L., et al., *Methods Enzymol.*, 230:44-57 (1994)}. The protein is not sensitive to endoglycosidase H, an enzyme which cleaves after the N-linked *N*-acetylglucosamine in high-mannose- and most hybrid-type oligosaccharides but does not cleave complex-type oligosaccharides {Trimbel, R.B., et al., *Anal. Biochem.*, 141:515-522 (1984)}. Lectin staining using *G. nivalis* agglutinin shows no evidence of terminal mannose characteristic for hybrid- and high-mannose-type glycosylation. In contrast, staining with ConA (mannose, *N*-acetylglucosamine, branched trimannosyl core) {Ogata, S., et al., *J. Biochem.*, 78:687-696 (1975)}, wheat germ agglutinin (*N*-acetylglucosamine), and BS-II (terminal *N*-acetylglucosamine) {Ebisu, S., et al., *Methods Enzymol.*, 50:350-354 (1978)} indicates the presence of terminal *N*-acetylglucosamine and internal mannose. Thus, there is evidence from the pattern of endoglycosidase sensitivity and lectin staining that gp18 is likely to be a biantennary complex-type glycoprotein.

gp18 is sensitive to endo-β-galactosidase. This enzyme cleaves between galactose and either *N*-acetylglucosamine or galactose when these saccharides occur in unbranched sequence {Scudder, P., et al., *J. Biol.*

Chem., 259:6586-6592 (1984)}. The presence of galactose was confirmed by BS-I lectin binding (FIG. 8). The presence of both *N*-acetylglucosamine and galactose was confirmed by high-performance anion-exchange chromatography with pulsed amperometric detection. The combination of *N*-acetylgalactosamine and galactose is usually found in O-linked carbohydrates {Hayes, B. K., et al., *J. Biol. Chem.* 268:16170-16178 (1993)}. Though it is possible that gp18 is both N and O glycosylated, *N*-acetylgalactosamine has also been reported to occur in complex-type N-linked glycosylation {Hayes, B. K., et al., *J. Biol. Chem.* 268:16170-16178 (1993)}.

We did not detect a monocistronic ~429-nt mRNA for gp18 by PCR using oligo(dT), a 5' sense primer, and template from a variety of sources, including infected cell lines and rat brain. In contrast, a 429-nt gp18 cDNA was readily amplified by using gene-specific primers and total RNA or poly(A)$^+$ RNA as a template. Northern (RNA) hybridization experiments with gp18-specific probes using total RNA or poly(A)$^+$ RNA from infected cells or rat brain detected only 1.5-, 2.8-, 3.5-, 6.1-, and 7.1-kb transcripts. Recent experiments confirmed that the 1.5- and 2.8-kb RNAs can serve as templates for *in vitro* translation of the gp18 (data not shown). These data suggest that gp18 is likely to be translated from one or more of the larger RNA transcripts.

The role of gp18 in the BDV life cycle remains to be determined. Though the virus has not been characterized morphologically, genetic analysis has characterized BDV as a member of the order *Mononegavirales* {Briese, T., et al., *Proc. Natl. Acad. Sci. USA* 91:4362-4366 (1994) and Cubitt, B., et al., *J. Virol.*, 68:1382-1996 (1994)}. In nonsegmented, negative-strand RNA viruses, the third gene usually directs expression of a matrix protein. Matrix proteins in members of the order *Mononegavirales* are not known to be

glycosylated; however, glycosylated matrix proteins that resemble gp18 in size and pI (≈10) have been found in other viral systems (*e.g.*, E1 in coronaviruses {Armstrong, J., et al., *Nature* (London), 308:751-752 (1984)}). Preliminary observations suggest that gp18 is present on the surface of the viral envelope. Monospecific antisera and monoclonal antibodies to gp18 precipitated viral particles and had neutralizing activity. In contrast, antibodies to p40 and p23 did not precipitate viral particles or neutralize infectivity (see Example 4 below). Preincubation of primary rabbit fetal glia (cells highly susceptible to BDV) with gp18 prevented infection. No such effect was observed with either p40 or p23. Last, gp18 and BDV particles compete for binding to a ≈100-kDa membrane protein present in cells susceptible to infection.

Expression of Recombinant P57

cDNAs representing the p57 ORF were amplified by RT-PCR using BDV (strain He/80)-rat brain RNA as template. The amplified p57 cDNA was subcloned into two plasmid vectors, pET21b (Novagen) and pSFV-1 (GIBCO BRL).

pET21b, a prokaryotic expression vector, was selected because it allows for tight control of protein expression, an important feature for expression of proteins toxic to host cells. The N-terminus of p57 contains a hydrophobic sequence that confers extreme toxicity to prokaryotic cells. Therefore, to facilitate the expression of p57, the first 152 N-terminal amino acids were excluded during the cloning. PCR amplified cDNA representing nucleotides 2697 to 3743 of p57 ORF (amino acids 153 to 503) was generated by using oligonucleotide primers designed with a 5′ restriction site (BamHl for sense primer; Xhol for antisense primer). The PCR product was cloned into pET21b at the BamHl and Xhol restriction sites, thus generating pET21b-BDV57$_{153-503}$. The pET21b-BDV57$_{153-503}$ plasmid was transformed into BL21

host cells and recombinant protein was expressed and purified by using protocols provided by the manufacturer.

An eukaryotic expression system, which allows for posttranslational modification, was selected for the expression of a recombinant protein more similar to native p57. pSFV-1 is a eukaryotic expression vector that can be used to generate a replication defective Semliki Forest virus (SFV) genomic RNA. The entire p57 ORF was PCR amplified and cloned into pSFV-1 prepared with 3' T-overhangs at the SmaI site, thus generating pSFV-BDV57. Transfection of pSFV-BDV57 transcripts into mammalian cells, results in overexpression of the posttranslationally processed p57 gene product.

EXAMPLE 3

ELISA for the Detection of Antibodies to Borna Disease Virus Proteins

We have expressed p40, p23 and gp18 as recombinant proteins and established a sensitive, specific ELISA for analyzing immunoreactivity to BDV. This assay system is more sensitive and rapid than methods currently employed for serologic diagnosis of infection such as Western blot, indirect immunofluorescent test (IFT) or immunoprecipitation.

This system provides a convenient tool for diagnosing disease, determining the prevalence of infection in animal and human populations and mapping the antigenic determinants for the immune response in infected hosts.

MATERIALS AND METHODS

Infection of Animals and Cultured Cells.

Six week old Lewis rats (Charles River) were infected intranasally with 6 x 10^4 focus forming units (ffu) of BDV strain He/80-1 {Carbone, K., et al., *J. Virol.*, 61:3431-3440 (1987) and Schneider, P. A., et al., *J. Virol.*, 68:63-68 (1994)}. C6 cells were persistently infected

with BDV He/80-1 (C6BDV) {Carbone, K. M., et al., *J. Virol.*, 67:1453-1460 (1993)}. Rabbit fetal glial cells were infected with BDV He/80-1 at a multiplicity of one ffu per cell then passaged once before use in IFT assays. BDV strain He/80 was originally isolated from infected horse brain, passaged twice in rabbits, three times in rabbit fetal glial cells, and twice in Lewis rats {Herzog, S., et al., *Med. Microbiol. Immunol.*, 168:153-158 (1980)}. He/80-1 was passaged four additional times in Lewis rats and used for infection of animals and cell lines.

Generation of Recombinant Proteins (recp40, recp23, and recp18)

Full length cDNAs encoding p40, p23 or gp18 were cloned into the prokaryotic expression vector pET15b (Novagen) for production of recombinant proteins. pBDV-40 in pcDNA II {McClure, M. A., et al., *J. Virol.*, 66:6572-6577 (1992)} was amplified using the primers p40Xho I (5'- CCCTCGAGGACCAAGATTT-3')(SEQ ID NO: 17) and Sp6 (20mer, Promega Corp., Madison, Wisconsin). pBDV-23 in pBluescript SKII+ {Thibault, K. J., M.S. thesis, University of California, Irvine (1992)} was amplified with the primers p24Nde I (5'- AGAATCATATGGCAACGCGACCATC-3')(SEQ ID NO: 18) and T7 (20mer Promega). Polymerase chain reaction was performed using Taq polymerase (Perkin-Elmer Cetus Corp., Norfolk, Connecticut) according to the manufacturer's protocol. Products amplified from pBDV-40 and pBDV-23 were phenol/chloroform extracted, precipitated and digested with BamH I and either Xho I (pBDV-40) or Nde I (pBDV-23) (Promega Corp., Madison, Wisconsin). pBDV-cp18 in pBluescript SKII+ (see Example 2 above) was digested with Xho I and BamH I. Digested fragments were purified by agarose gel electrophoresis (USB, USBioclean, Cleveland, Ohio) and cloned into pET15b (Novagen Corporation, Madison, Wisconsin). Protein expression in plasmid containing

Escherischia coli cells was induced by addition of isopropyl-β-thiogalactopyranoside (1 mM) for 3 hours at 37°C. Proteins (recp40, recp23, and recp18) were purified by nickel-chelate affinity chromatography according to manufacturer's instructions (Novagen Corp.). Purification was assessed by SDS-PAGE and antigenicity was confirmed by Western blot using sera from infected rats. Proteins were dialyzed against 150 mM NaCl and 2.5 mM $CaCl_2$ and digested with biotinylated thrombin (1 unit/mg recombinant protein, Novagen Corp.) overnight at room temperature. Thrombin was removed using streptavidin-agarose (Novagen Corp.) according to manufacturer's protocol. Protein concentrations were estimated by BioRad protein assay according to manufacturer's instructions.

Antibodies to BDV and recombinant BDVproteins

Sera were collected from infected rats at time of sacrifice or by tail bleeding at 2-week intervals after inoculation with BDV. Antibodies to recp40 and recp23 were each produced in two rabbits. Animals were injected subcutaneously (s.c.) with 25 μg of protein in Freund's complete adjuvant and then boosted 3 weeks later s.c. with 25 μg of protein in Freund's incomplete adjuvant. After 6 weeks some animals received an additional s.c. injection of 25 μg protein in Freund's incomplete adjuvant. Blood was collected at 2-week intervals during weeks 7 through 14 for detection of antibodies by Western blot and ELISA.

Indirect Immunofluorescent Test (IFT)

Rabbit fetal glial cells were processed for titration of serum antibodies against BDV using the immunohistochemical methods of Pauli et al. {Pauli, G., et al., *Zbl. Vet. Med. [B]* 31:552-557 (1984)}. Briefly, infected and noninfected cells were fixed with 4% formaldehyde in PBS, permeabilized with 1% Triton X-100

in PBS and blocked with 1% fetal bovine serum (FBS) in PBS. After incubation with sera diluted in 1% FBS in PBS, cells were incubated with fluorescein-conjugated goat anti-rat IgG and IgM or goat anti-rabbit IgG (Sigma Chemical Co., St. Louis, Missouri) diluted 1:200 in 1% FBS in PBS and then examined by fluorescent microscopy. The IFT titer for each serum was determined to be the endpoint dilution at which specific inununoflourescence was detected.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), Western blot (WB) and Immunoprecipitation (IP)

For WB, lysates from infected and noninfected C6 cells were prepared according to Bause-Niedrig, et al. {Bause-Niedrig, I., M. et al., *Vet. Immunol. Immunopathol.*, 31:361-369 (1992)}. Proteins from these lysates (30 μg) and recombinant BDV proteins (250 ng) were subjected to 12% SDS-PAGE {Laemmli, U. K., et al., *J. Mol. Biol.*, 80:575-581 (1973)} and then transferred to nitrocellulose membranes (Schleicher & Schuell, Keene, New Hampshire) {Towbin, H., et al., *Proc. Natl. Acad. Sci. USA*, 76:4350-4354 (1979)}. Membranes were incubated at room temperature first with WB-diluent (0.5% nonfat dry milk (Carnation Company, Los Angeles, California) and 0.05% Tween-20 (Fisher Scientific, Raleigh, North Carolina) in TBS (tris balanced saline, 50 mM Tris-HCl pH 7.5 and 150 mM NaCl)) for one hour, then overnight with various dilutions (1:10 to 1:2,000) of rat sera or monospecific rabbit sera in WB-diluent. Membranes were washed 3 times in TBS, incubated for 2 hours with the appropriate secondary antibody (horseradish peroxidase-conjugated goat anti-rat IgG and IgM or goat anti-rabbit IgG, Sigma Chemical Co., St. Louis, Missouri) diluted 1:500 in WB-diluent, washed 5 times in TBS and then incubated with hydrogen peroxide and 4-chloro-1-naphthol (Pierce Chemical Company, Rockford, Illinois) according to manufacturer's

instructions. Methods for synthesis and analysis of radiolabeled BDV proteins and iminunoprecipitation have been described {Lipkin, W. I., et al., *Proc. Natl. Acad. Sci., USA*, 87:4184-4188 (1990)}. Briefly, plasmid clones pBDV-gp18, pBDV-23 and pBDV-40 were linearized and used as template for *in vitro* transcription and translation of [^{35}S] methionine-labeled proteins. After precipitation with rat or rabbit sera and protein A-sepharose (Sigma Chemical Co., St. Louis, Missouri), proteins were analyzed by SDS-PAGE and autoradiography.

ELISA

Ninety-six well, Immulon I microtiter plates with lids (Dynatech Laboratories, Chantilly, Virginia) were coated overnight at 37°C with 10 ng of recombinant protein per well in 100 µl of borate buffer (100 mM boric acid, 50 mM sodium borate and 75 mM sodium chloride, pH 8.4). Plates were washed three times with washing buffer (0.05% Tween-20 in PBS) and incubated for 1 hour at 37°C with ELISA-diluent (0.5% bovine serum albumin (BSA) fraction V (USB) in washing buffer). Two-fold serial dilutions of sera were prepared in ELISA-diluent; 100 µl of sera diluted from 1:250 to 1:500,000 was then added to each well and incubated for 2 hours at 37°C. Plates were washed three times with washing buffer. Next, 100 µl of horseradish peroxidase-conjugated goat anti-rat IgG and IgM (Sigma Chemical Co., St. Louis, Missouri) diluted 1:5,000 in ELISA-diluent were added to each well and incubated for 1 hour at 37°C. After washing the plates five times, 100 µl of substrate solution was added to each well. Substrate solution consisted of 9.9 ml of 100 mM sodium acetate adjusted to pH 6.0 with 100 mM citric acid, 100 µl of 10 mg of 3,3',5,5'-tetramethylbenzidine (Sigma Chemical Co., St. Louis, Misouri) per ml in dimethyl sulfoxide and 1.5 µl of 30% hydrogen peroxide (Fisher Scientific, Raleigh, North Carolina). After incubation in the dark at room temperature for 30

minutes, the reaction was stopped by the addition of 50 μl of 25% sulphuric acid (Sigma Chemical Co., St. Louis, Missouri) to each well. The absorbance at 450 nm was determined for each well using a microplate reader (Molecular Devices, Thermo max, Menlo Park, California). Negative control wells, without primary antisera, were used for calibration. The ELISA titer for each serum was defined as the endpoint dilution that yielded an optical density of 0.3.

RESULTS

The figures below present some of the results:

FIG. 11. Western blot analysis of native and recombinant proteins with monospecific antisera to recombinant proteins and sera from infected rats. Recombinant viral proteins and lysates from infected C6BDV or noninfected C6BDV cells were size-fractionated and screened by Western blot. A) Sera from infected and noninfected rats were used to detect native or recombinant proteins. Lane 1, C6BDV lysate; lane 2, recp40; lane 3, recp23; lane 4, recp18; lane 5, C6 lysate; lane 6, recp40, recp23 and recp18. Lanes 1-4 were treated with serum from infected rat; lanes 5 and 6 were treated with serum from noninfected rat. B) Monospecific antisera were used to detect BDV-specific proteins. C6BDV lysates (lanes 1-3) and C6 lysates (lanes 4 and 5) were incubated with: lanes 1 and 4, serum from infected rat; lane 2, anti-p40 rabbit serum; lane 3, anti-p23 rabbit serum; and lane 5, pooled anti-p40 and anti-p23 sera.

FIG. 12. ELISA of infected rat serum reacted with recp40. ELISA was performed with 10 ng/well recp40 or BSA as described in Materials and Methods. Circles, recp40 and serum from chronically infected rat; squares, recp40 and serum from noninfected rat; triangles, BSA and serum from chronically infected rat.

FIG. 13. Timecourse for appearance of antibodies to BDV-proteins. Sera were collected at different times post-infection and assayed by ELISA for antibodies to (A) recp40; (B) recp23; and (C) recp18. Error bars represent standard error of the mean. Number of animals analyzed at each time point: <4 wks, 15; 5 wks, 6; 6 wks, 12; 8 wks, 4; 10 wks, 5; and 15 wks, 9.

Production of recombinant viral proteins and monospecific antisera to recombinant viral proteins

Full length coding sequences for p40, p23 and gp18 were expressed in *Escherischia coli* and recombinant proteins were purified. The yield of protein in 100 ml of bacterial culture was: recp40, 1 mg; recp23, 500 μg; and recp18, 50 μg. Recombinant proteins were analyzed by SDS-PAGE. A predominant band of the expected molecular weight was observed for each protein and tested for antigenicity by WB using sera from BDV-infected and noninfected rats (FIG. 11A). Recombinant proteins were detected by sera from BDV-infected rats but not by sera from noninfected rats. Recombinant proteins, recp40 and recp23 were used to produce antibodies in rabbits. The production of antibodies was monitored by ELISA. Rabbits were sacrificed when the ELISA titer reached 1:500,000 (week 16 of immunization). The specificity of the antisera was then tested by WB using lysates from infected cells and recombinant proteins (FIG. 11B). Antisera were monospecific: rabbits immunized with recp40 produced antibodies that reacted only with p40 and recp40; rabbits immunized with recp23 produced antibodies that reacted only with p23 and recp23. At week 16 of immunization, the antisera were also titered by IFT. Antisera to recp40 and recp23 had IFT titers of 1:50,000 and 1:100,000, respectively.

Specificity and sensitivity demonstrated in the BDV-ELISA systems

In order to establish a sensitive and specific ELISA for all three recombinant BDV proteins, the optimal antigen concentration was determined by checkerboard titration of positive and negative sera versus various antigen concentrations. For each protein, the concentration that resulted in the most linear response was 10 ng/well. The sensitivity of the ELISA system for each recombinant protein was established using sera from infected rats known to be reactive by IFT, IP and WB. For each of the proteins, 100% of sera that had been found to be positive by other methods were also positive by ELISA. Specificity was tested using sera from 15 noninfected rats. ELISA for each protein proved to be highly specific for detection of antibodies to BDV proteins: recp40-ELISA with noninfected rat sera showed 80% specificity at 1:500 dilution or 100% specificity at 1:2,000, recp23-ELISA showed 93% specificity at 1:250 and 100% specificity at 1:1,000, recp18-ELISA showed 100% specificity at 1:250. Figure 12 shows a representative ELISA using recp40 as target antigen. Various dilutions of sera from chronically infected and noninfected rats were tested with 10 ng of recombinant protein or BSA per well in comparison with BSA. No nonspecific background reactivity was observed at serum dilutions of 1:500 or higher (FIG. 12). Results were similar when recp23 and recp18 were used as target antigen.

Analysis of immunoreactivity to viral proteins by IFT WB, IP and ELISA in sera from infected rats.

Adult rats infected intranasally with BDV did not display abnormal behaviors prior to the fourth week post-infection (predisease, PD). Four to six weeks post-infection, in the acute phase of disease (AD), animals had hyperactivity, weight loss, disheveled fur, dystonic posture and hindlimb paresis. Eight to fifteen weeks

post-infection, signs of disease stabilized: there was no additional weight loss, hyperactivity diminished and paresis did not progress. This chronic phase of the disease (CD) persisted for the life of the animals. Sera was collected from adult-infected rats between 3 and 15 weeks after infection with BDV, and analyzed for the presence of antibodies to viral proteins using four different methods: IFT, WB, IP and ELISA (Table 3).

TABLE 3 Detection of BDV-specific antibodies in sera from infected rats by different methods

Serum	WB			IP[a]			Reciprocal ELISA titer[b]			Reciprocal IFT titer
	recp40	recp23	recp18	p40	p23	p18	recp40	recp23	recp18	
PD (3–4 wk pi[c]; n = 15)	−	−	−	−	−	−	2,388 ± 256	904 ± 181	163 ± 5[d]	<10
AD (4–6 wk pi; n = 18)	+	+	−	+	+	−	3,217 ± 829	2,644 ± 20	279 ± 19	20–200
CD (10–15 wk pi; n = 14)	+	+	+	+	+	+	291,889 ± 56,590	76,527 ± 19,309	4,680 ± 1,467	10,000–20,000

[a] In vitro-translated proteins.
[b] Values are mean ± standard error of the mean titer.
[c] pi, postinfection.
[d] Nonspecific. Value below the level of specificity of the recp18 ELISA (1:250).

IFT allowed detection of antibodies to BDV in both AD rats and CD rats. In AD rats, the titer was between 1:20 and 1:200, whereas in CD rats, the titer was between 1:10,000 and 1:20,000. Sera from PD rats were not reactive by IFT. WB using lysates from infected cells or recombinant proteins, and IP using proteins translated *in vitro* yielded identical results: sera from CD animals were reactive with p40, p23 and gp18; sera from AD rats detected only p40 and p23; sera from PD rats did not react with p40, p23 or gp18. ELISA detected antibodies reactive with p40, p23 and gp18 in sera from all CD and AD rats (Table 3). In PD rats, ELISA only detected antibodies reactive with p40 and p23; immunoreactivity with gp18 was below specificity (Table 3).

The timecourse for the appearance of antibodies to BDV-proteins in sera was determined by ELISA. Sera collected at regular intervals from adult-infected rats were tested in the recp40, recp23 and recp18 ELISA systems. Titers of antibodies to all three proteins increased throughout the period of observation from weeks 4 to 15 post infection (FIG. 13).

DISCUSSION

Three recombinant BDV proteins, recp40, recp23 and recp18, were expressed and used as immunogens for production of monospecific sera in rabbits. Two of these antisera, directed against recp40 and recp23, are reported here; antisera to recp18 are described in Example 4 below. These three recombinant proteins were detected by sera from infected rats (FIG. 11A) and by monoclonal antibodies to purified native proteins. Monospecific antisera to the recombinant proteins were immunogen-specific as determined by WB (FIG. 11B) and detected proteins in infected cells by IFT.

ELISA systems were established, based on recombinant proteins, that have several advantages over methods currently used for detection of BDV-specific antibodies

including IFT, WB and IP. Although IFT is widely accepted as a method for diagnosing BDV infection and titering antibodies to the virus, it has two disdavantages. First, IFT does not define the viral protein(s) responsible for immunoreactivity. Second, as shown here, IFT titers are 10-100 fold less sensitive than ELISA for detection of antibodies to p40 or p23. This relative insensitivity resulted in failure of IFT to show evidence of infection in PD rats (Table 3). WB and IP allowed detection of antibodies to individual viral proteins but were also less sensitive than ELISA. Sera from PD rats were not reactive by either WB or IP.

For diagnostic purposes, the recp40-ELISA is the most sensitive method for detection of antibodies in infected animals. Antibodies to recp40 were present prior to disease onset and had higher titers than antibodies to recp23 or recp18. Although the recp23-ELISA was also positive in PD and AD rats, the recp18-ELISA was not. Because high titer antibodies to gp18 only appear in chronic disease, the recp18-ELISA may be used to estimate the duration of infection. Low antibody titers to recp18 are not due to the lack of glycosylation on this recombinant protein because similar ELISA titers were found with native gp18 antigen. Failure to produce high titer antibody response to recp18 may be due to lower levels of expression of this protein than p40 or p23.

Growing recognition that BDV has a broader species and geographic range than previously appreciated suggests the importance of designing sensitive, reliable assays for infection. The ELISA systems described here, provide inexpensive, rapid methods for BDV-serology. In contrast to IFT, WB and IP, which require at least 2 days for completion and are not well suited to screening multiple samples, ELISA allows analysis of hundreds of sera in several hours with only minimal equipment. Plates coated with these proteins have been stable in ELISA for up to

one month at room temperature and thus are practical for use in remote laboratories. In addition to serving as a tool for clinical diagnosis and epidemiology of Borna disease infection, the BDV ELISA is a useful tool for studies in immunopathogenesis and virus biology. For example, applicants have mapped antigen binding sites on p40 and p23 by ELISA using sera from infected animals and monoclonal antibodies to BDV proteins.

Dependent on the population studied and the methods used for analysis (WB, IP or IFT), the prevalence of antibodies reactive with BDV proteins in patients with neuropsychiatric disorders has been estimated to be between 4% and 23% {Bode, L., *In* W. I. Lipkin and H. Koprowski (ed.), Borna Disease. Springer-Verlag, Heidelberg, in press (1995)}. Variability between laboratories could be due to differences in populations analyzed, antigen preparations or experimental technique. The BDV ELISA based on recombinant proteins provides a standardized method for investigating human immunoreactivity to this neurotropic infectious agent.

EXAMPLE 4

Neutralizing Antibodies in BDV Infected Animals

We examined the timecourse for the development of neutralization activity and the expression of antibodies to individual BDV viral proteins in sera of infected rats. The appearance of neutralizing activity correlated with the development of immunoreactivity to gp18, but not p40 or p23. Monospecific and monoclonal antibodies to native gp18 and recombinant non-glycosylated gp18 were also found to have neutralizing activity and to immunoprecipitate viral particles or subparticles. These findings suggest that gp18 is likely to be present on the surface of the viral particles and to contain epitopes important for virus neutralization.

Antibodies to p40 and p23 (soluble antigens) are readily detected in both sera and cerebrospinal fluid

(CSF) of naturally and experimentally infected animals {Ludwig, H., et al., *Progr. Med. Virol.*, 35:107-151 (1988); Ludwig, H., et al., *Arch. Virol.*, 55:209-223 (1977) and Ludwig, H., et al., *Med. Microbiol. Immunol.*, 163:215-226 (1977)}. Antibodies to gp18, a membrane-associated glycoprotein (previously described as 14.5 kDa), have been reported less frequently {Ludwig, H., et al., *Progr. Med. Virol.*, 35:107-151 (1988) and Rubin, S. A., et al., *J. Virol.*, 67:548-52 (1993)}. Although neutralization activity has been found in sera of animals infected with BDV {Danner, K., et al., *Zbl. Vet.-Med. [B]*, 25:345-355 (1978); Hirano, N., et al., *J. Gen. Virol.*, 64:1521-1530 (1983); Ludwig, H., et al., *Progr. Med. Virol.*, 35:107-151 (1988) and Ludwig, H., et al., *Arch. Virol. [Suppl]* 7:111-133 (1993)}, the antibodies responsible for neutralization activity have not been investigated. An enzyme-linked immunosorbent assay (ELISA) based on recombinant BDV proteins has been established in Example 3 above, that provides a sensitive method for detection of antibodies to gp18. We find that the appearance of neutralizing antibodies in infected rats correlates with immunological reactivity to gp18. Furthermore, monospecific and monoclonal antibodies (MAbs) directed against gp18 neutralize BDV infectivity and immunoprecipitate viral particles or subparticles.

MATERIALS AND METHODS

BDV infected animals: Sixty-thousand focus forming units (ffu) of BDV strain He/80-1 {Carbone, K. M., et al., *J. Virol.*, 61:3431-3440 (1987); Herzog, S., et al., *Med. Microbiol. Immunol.*, 168:158-8 (1980) and Schneider, P. A., et al., *J. Virol.*, 68:63-68 (1994)} were used to intranasally (i.n.) infect each of seventy 6-week old Lewis rats. Rats were observed at three days intervals for weight loss, ruffled fur or postural abnormalities consistent with acute disease. Sera were collected at time of

sacrifice. Under metofane anesthesia, rats were perfused with buffered 4% paraformaldehyde; brains were fixed overnight in perfusate at 4°C. Twenty-micron sagittal sections were collected onto gelatin coated slides and stained with hematoxylin and eosin. Inflammation was scored using the scale of Stitz, Sobbe and Bilzer {Stitz, L., et al., *J. Virol.*, 66:3316-23 (1992)}.

Virus titration and neutralization assay.

Viral infectivity in 20% brain homogenates was determined using the method of Pauli et al. {Pauli, G., et al., *Zbl. Vet.-Med. [B]* 31:552-557 (1984)}. Virus neutralization was performed using a modification of Danner et al. {Danner, K., et al., *Zbl. Vet.-Med. [B]*, 25:345-355 (1978)}. Briefly, 50 ffu of BDV were incubated with serial dilutions of antibodies or sera for one hour at 37°C, added to rabbit fetal glial cells and incubated for 5 days. Sera was heat inactivated at 56°C for 30 minutes. In selected assays, mouse complement (1:50) (Sigma Chemical Co., St. Louis, Missouri) was added to the virus concurrent with the addition of MAbs to determine the effects of complement on neutralization activity. The dilution of serum or antibody required to reduce the number of ffu by 50% was defined as the neutralization titer (NT_{50}). As controls for each neutralization assay, rabbit fetal cells were exposed to medium without virus, treated with virus in medium alone (no antibodies), or treated with virus incubated with sera from normal rats. Pilot studies showed that approximately 8% of normal rat sera interfered with BDV infectivity at dilutions up to 1:16. Therefore, sera were considered to be neutralizing only if the NT_{50} exceeded 1:32. Supernatant from nonproducing myeloma cell lines as well as monoclonal antibodies directed against BDV-p23 (24/36F1) and BDV-p40 (38/17C1) {Thiedemann, N., et al., *J. Gen. Virol.*, 73:1057-1064 (1992)} were found to neutralize infectivity at dilutions of 1:2.

Thus, monoclonal antibodies were considered to be neutralizing only if the NT50 exceeded 1:4.

Preparation of proteins (recp40, recp23, recp18 and gp18):

Plasmids encoding p40 {pBDV-40 disclosed in McClure, M. A., et al., *J. Virol.*, 66:6572-6577 (1992)}, p23 {pBDV-23 disclosed in Thibault, K. J., M.S. thesis; University of California, Irvine (1992)} and gp18 {pBDV-gp18 disclosed in Kliche, S. *et al., J. Virol.*, 68:6918-6923 and Example 2 above} were subcloned (see Example 3 above) into the prokaryotic expression vector petl5b (Novagen, Madison, Wisconsin). Recombinant proteins (recp40, recp23 and recp18) were expressed in *Escherichia coli* and purified according to manufacturer's protocol (Novagen, Madison, Wisconsin). Purity and antigenicity were assessed by SDS-PAGE and Western blot analysis using sera from infected rats. Native, glycosylated gp18 was prepared from infected rat brain as described previously {Schädler, R., et al., *J. Gen. Virol.*, 66:2479-2484 (1985)}.

Enzyme-linked immunosorbent assay (ELISA):

ELISA was performed as described in Example 3 above. Briefly, plates coated with recombinant protein were incubated with serially diluted sera or MAbs. Bound horseradish peroxidase (HRPO)-coupled secondary antibody (goat anti-mouse F'ab-HRPO, goat anti-rat IgG and IgM HRPO; Sigma Chemical Co.) was quantified on a microplate reader (Thermo max, Molecular Devices, Menlo Park, California) using the chromagen 3,3'-5,5' Tetramethylbenzidine (Sigma Chemical Co.). The ELISA endpoint titer was defined as the serum or antibody dilution that generated an optical density of 0.3.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), Western blot and Immunoprecipitation (IP):

Recombinant or native BDV proteins were subjected to SDS-PAGE {Laemmli, U. K., et al., *J. Mol. Biol.*, 80:575-581 (1973)} and transferred to nitrocellulose (Schleicher & Schuell, Inc., Keene, New Hampshire) or Immobilon-N membranes (Millipore Corp., Bedford, Maryland) {Towbin, H., et al., *Proc. Natl. Acad. Sci. USA*, 76:4350-4354 (1979)}. Membranes were blocked and incubated with primary antibody as described in Example 3 above. After incubation with secondary antibody (goat antimouse IgG-alkaline phosphatase [AP], goat anti-rat IgG-AP or goat anti-rat IgG and IgM-HRPO, Sigma Chemical Co., St. Louis, Missouri), immune complexes were visualized using Western Blue (Promega, Madison, Wisconsin) for AP or chemiluminescence (ECL kit, Amersham, Arlington Heights, Illinois) for HRPO according to manufacturer's instructions. gp18 or recp18 were precipitated using sera from infected rats, monospecific antibodies or MAbs and Protein A-Sepharose (Pharmacia Biotech Inc., Piscataway, New Jersey) as described by Persson, H., et al. {Persson, H., et al. *Science*, 225:687-693 (1984)} then assayed by Western blot.

Monoclonal antibodies:

MAbs to gp18 were generated according to Thiedemann et al. {Thiedemann, N., et al., *J. Gen. Virol.*, 73:1057-1064 (1992)}. Briefly, Balb/c mice were immunized intraperitoneally (i.p.) with 5 μg of gp18 in complete Freund's adjuvant. Three and 6 weeks after the initial immunization, mice were boosted i.p. with 5 μg of gp18 in incomplete Freund's adjuvant. Four days before fusion of spleen cells with the mouse myeloma cells X63-Ag8.653 {Kearney, J. F., et al., *J. Immunol.*, 123:1548-1550 (1979)}, mice were boosted intravenously with 15 μg of gp18. All hybridomas were initially screened for reactivity to gp18

by ELISA. Tissue culture supernatants from positive hybridomas were concentrated by ammonium sulfate precipitation {Jonak, Z. L., p. 405-406, *In* R. H. Kennett, T. J. McKean, and K. B. Becktol (ed.), "Monoclonal antibodies, Hybridomas: A new dimension in biological analyses", Plenum Press, New York (1982)} and tested by Western blot and IP for reactivity with gp18 and recp18. The immunoglobulin isotype was determined using an agglutination isotyping kit (Serotec, Oxford, England) according to manufacturer's instructions. Monoclonal antibody, 24/36F1 directed against BDV-p23 {Thiedemann, N., et al., *J. Gen. Virol.*, 73:1057-1064 (1992)}, was used as a negative control in Western blot and IP experiments.

Generation of polyclonal sera against recp18 protein:

To produce antibodies against recp18, two 2-month old Lewis rats were injected subcutaneously (s.c.) with 25 μg of protein in Freund's complete adjuvant and boosted 3 weeks later with 25 μg of protein s.c. in Freund's incomplete adjuvant. After 6 weeks, animals received i.p. injections of 25 μg protein in phosphate buffered saline (PBS) with 20 μg lipopolysaccharide (*S. typhimurium*, Difco, Detroit, Michigan) at two-week intervals for a total of three injections. Serum was collected every two weeks during weeks 7 through 14 for analysis by ELISA and Western blot and for determination of neutralization titer. Mouse antibodies to native gp18 have been described in Example 2 above.

Affinity adsorption of BDV-specific serum-antibodies:

Antibodies that bound to recp23 and recp40 were sequentially removed from serum of an infected rat according to Crabb et. al. {Crabb, B. S., et al., *Virology*, 190:143-154 (1992)}. Serum (D2) from an adult-infected Lewis rat (15 weeks post intranasal infection), was diluted 1:10 in TBS (tris balanced saline, 50 mM Tris pH

7.4 and 100 mM NACl) and incubated overnight at 4°C with membrane-bound recp23. The anti-recp23 antibody-depleted serum (D2 Δαrecp23) was removed, the membrane was washed with TBS and adsorbed anti-recp23 antibodies were eluted (recp23 eluant) by incubation with 1 ml of 0.1 M glycine, 0.15 M NaCl pH 2.7 for 3 minutes. The pH of the eluant was adjusted by addition of 300 μl of 10 mM Tris HCl pH 7.5. The anti-recp23 antibody-depleted serum was then incubated with membrane-bound recp40 (D2 Δαrecp23, Δαrecp40) and purified as before (recp40 eluant). Antibody depletion from serum and antibody elution from membrane-bound proteins was monitored by Western blot and ELISA. At each step during the purification, antibody-depleted sera and eluted antibodies were analyzed for neutralizing activity. Antibodies to gp18 or recp18 were also adsorbed (D2 Δαgp18, D2 Δαrecp18) and eluted (gp18 eluant, recp18 eluant) by this method. These adsorption and elution experiments were repeated using serum (B3) from an additional adult-infected rat (15 week post intranasal infection).

IP of BDV particles or sub particles and analysis by reverse transcription polymerase chain reaction (PCR):

Forty-thousand ffu of BDV in a volume of 200 μl were treated with 50 μg/ml of DNase I and RNase A (Boehringer Mannheim Corp., Indianapolis, Indiana) for 30 minutes at 37°C then incubated for 2 hours at room temperature with 100 μl of one of the following: (1) serum from acutely or chronically infected rats at 1:10 dilution in PBS; (2) purified serum-antibodies at 1:10 dilution; (3) mouse anti-gp18 sera or rat anti-recp18 sera at 1:20 dilution; or (4) monoclonal antibodies against gp18 at 1:5 dilution. Next, 100 μl of 1 mg/ml Protein A-Sepharose (Pharmacia, Puscataway, New Jersey) in PBS was added, and the mixture was incubated overnight at 4°C. The Protein A-Sepharose-antibody-virus complex was washed three times in PBS then resuspended in 100 μl

water. Total RNA was extracted {Chomczynski, P., et al., *Anal. Biochem.*, 162: 156-159 (1987)} and used for RT-PCR amplification of a 693 nucleotide region of the viral genome (nucleotide 753 to 1446) according to Schneider et al. (primer 7 and primer 9) {Schneider, P. A., et al., *J. Virol.*, 68:63-68 (1994)}. PCR products were analyzed by agarose gel electrophoresis. PCR products were cloned and sequenced to confirm that they represented the predicted region of the genomic RNA {Schneider, P. A., et al., *J. Virol.*, 68:63-68 (1994)}. Negative controls for RT-PCR included the omission of virus from immunoprecipitation reactions and the use of genomic sense primers during first strand cDNA synthesis.

RESULTS

The following figures present part of the results:

FIG. 14. Timecourse for the appearance of antibodies to BDV proteins in sera from individual rats after i.n. infection. (A) Neutralization activity in sera from BDV-infected rats at three timepoints (5, 10 and 15 weeks post-infection). Each serum is represented by a circle. Bars indicate mean neutralization titer for each group (5, 10 or 15 weeks post-infection). Asterisk represents sera with neutralization titer less than or equal to 1:16. (B) Plot of mean recp18 ELISA titers (open columns) with neutralization titers (hatched columns) at three time points (5, 10 and 15 weeks post-infection). Sera analyzed were the same as those in panel A. Mean values for neutralization activity were determined as described in FIG. 14A. Arrows indicate threshold for significance in neutralization assay (1:32) and recp18 ELISA (1:250). These values were selected because normal rat sera reacted in the neutralization assay and reccp18 ELISA at titers of 1:16 and 1:125, respectively. (C) Timecourse for the appearance of antibodies to recp40, recp23, and gp18 by Western blot analysis. Proteins were size-fractionated by SDS-PAGE

and transferred to nitrocellulose membranes. Membranes were incubated first with sera and then with horseradish peroxidase-coupled goat anti-rat IgG. Bound secondary antibody was detected by chemiluminescence. Results shown are from serum of one representative animal at several different timepoints post BDV infection (p.i.).

FIG. 15. Monoclonal antibody (MAb) detection of gp18. A) Immunoprecipitation of gp18 with MAbs. gp18 was first incubated with MAbs or sera from infected or noninfected rats, then precipitated with Protein-A Sepharose, size-fractionated by 12% SDS-PAGE and transferred to Immobilon-N membranes. Precipitated gp18 was visualized with rat anti-recp18 sera, goat anti-rat IgG-AP, and Western Blue. Lanes 1, serum from infected rat (15 week p.i.); 2, serum from noninfected rat; 3, MAb 14/29A5; 4, MAb 14/26B9; 5, MAb 14/8E1; 6, MAb 14/13E10; 7, MAb 14/18H7; 8, MAb 24/36F1 (MAb directed against the BDV 23 kDa protein, negative control); 9, no antibody. Arrow indicates gp18; H and L represent heavy and light chains of immunoglobulin, respectively. B) MAbs were analyzed for binding to native gp18 in Western blot. gp18 was separated on 12% SDS-PAGE and transferred to an Immobilon-N membrane. Strips were incubated with MAbs or sera from infected or noninfected rats. Bound antibodies were detected with alkaline phosphatase conjugated goat anti-rat IgG or goat anti-mouse Fab-specific and Western Blue substrate. Lanes: 1, serum from infected rat (15 week p.i., D2); 2, serum from noninfected rat; 3, MAb 14/29A5; 4, MAb 14/26B9; 5, MAb 14/8E1; 6, MAb 14/13E10; 7, MAb 14/18H7; and 8, MAb 24/36F1 (MAb directed against the BDV 23 kDa protein, negative control). Molecular weight markers (10^3 Da) are shown at the right.

FIG. 16. Neutralization profile of sera and MAbs. BDV (50 ffu) was preincubated with serial dilutions of serum or MAb and then added to ten thousand rabbit fetal glial cells. After four days of incubation, the infected cells were visualized as described in Pauli et al.

{Pauli, G., et al., *Zbl. Vet.-Med. [B]* 31:552-557 (1984)}. The number of infected cell-foci per well was counted. (A) Serum from noninfected rat. (B) serum from infected rat (15 week p.i., D2). (C) MAb 14/13E10. (D) MAb 14/29A5.

FIG. 17. Precipitation of BDV using sera from infected rats, monospecific rat antisera to recp18 and monoclonal antibodies (MAbs) to gp18. Virus was treated with nucleases to eliminate nucleic acid not contained within virions then immunoprecipitated with sera or MAbs and Protein A-Sepharose. RNA was extracted and subjected to RT-PCR to amplify a 693 nucleotide viral genomic sequence. PCR-products were visualized in an ethidium bromide-stained 1% agarose gel. (A) Precipitation of BDV with sera from infected rats. Lanes: 1, serum from infected rat, 15 week p.i.; 2, serum from infected rat, 5 week p.i.; 3, serum from infected rat, 15 week p.i., no BDV; 4, serum from infected rat,15 week p.i., genome sense primer used for first strand cDNA synthesis. (B) Precipitation of BDV by monospecific antisera to recp18 and MAbs to gp18. Lanes: 1, monospecific rat antisera to recp18; 2, MAb 14/13E10; 3, MAb 14/29A5. DNA markers (basepairs) are shown at the right.

Timecourse of disease and appearance of antibodies to BDV in infected rates:

Rats developed Borna disease (BD) within 5 weeks post infection. The acute phase of the disease, 4-8 weeks post infection, was associated with marked weight loss, disheveled fur, dystonic posture, hind limb paresis and paralysis, mortality of 35%, and prominent inflammatory cell infiltrates in the brain. In the chronic phase of disease, 10-15 weeks post-infection, signs of disease stabilized and inflammation receded. Virus titers in the brains of animals acutely (5 weeks p.i.) and chronically infected (15 weeks p.i.) were $2.4\pm0.4\times10^5$ ffu/ml and $4.4\pm0.2\times10^4$ ffu/ml, respectively.

Sera were monitored for virus neutralization activity (FIG. 14A, B and C) and the presence of antibodies reactive with recp40, recp23, recp18 or native gp18 in Western blot (FIG. 14C) and ELISA. Neutralization activity was first detected in sera (28% of the animals) at 5 weeks p.i. By week 15 p.i., all sera had neutralization activity with a mean titer of 1:977±246. Antibodies to recp18 were first detected by ELISA at week 5 p.i. and showed a marked increase in titer by 15 weeks p.i. (1:4,610±1,463) (FIG. 14B). In contrast, antibodies reactive with recp40 and recp23 were detected by ELISA within 4 weeks of infection, reached a titer greater than 1:20,000 by 8 weeks p.i. and remained elevated through 15 weeks p.i. (see Example 3 above). Antibodies reactive with recp40 and recp23 were detected by Western blot between weeks four and five p.i., whereas antibodies to gp18 were detectable only after week 10 p.i. (FIG. 14C).

Affinity adsorption of neutralizing sera:

To determine whether the presence of antibodies to gp18 correlate with neutralization activity, two rat sera (D2 and B3, 15 weeks p.i.), were tested in the neutralization assay after successive depletions of antibodies to individual BDV-proteins. Antibodies to BDV-specific proteins were removed from D2 rat serum by adsorption with membrane-bound protein. The efficiency of antibody depletion from serum was monitored by Western blot and ELISA. Prior to adsorption, the titers to recp40 and recp23 were each greater than 1:20,000. Following adsorption with recp23, the titer to recp23 decreased to 1:200. After adsorption with recp40, the titer to recp40 decreased to 1:150. Eluted antibodies were reactive by ELISA with the proteins used for adsorption: recp23 eluant titer, 1:5,000; recp40 eluant titer, 1:15,000. Serum antibodies remaining after adsorption, and eluted antibodies, were then tested for

neutralizing activity. The neutralization titer of the D2 serum (NT_{50} 1:1,000-1,500) did not change after adsorption with recp23 and recp40 antigens (D2 Δαrecp23, Δαrecp40) (Table 4). Antibodies eluted from proteins recp40 (recp40 eluant) and recp23 (recp23 eluant) had no neutralization activity (Table 4). In contrast, the NT_{50} of the D2 serum decreased from 1:1,000-1,500 to 1:600-700 after adsorption with recp18 (D2 Δαrecp18) and to 1:160-200 after adsorption with gp18 (D2 Δαgp18) (Table 5). The neutralization titers of antibodies eluted from recp18 (recp18 eluant) and gp18 (gp18 eluant) were 1:60-100 and 1:240-400, respectively (Table 4). Similar results were obtained with serum from rat B3.

TABLE 4 Characterization of serum antibodies

Serum	Reciprocal NT	IP-RT-PCR[a]	Reciprocal rp18 ELISA titer
Chronic (15 wk p.i. [D2])	1,000–1,500	+	4,300–5,000
D2 Δαrecp23, Δαrecp40[b]	1,000–1,500	+	4,000–5,000
D2 Δαrecp18[b]	600–700	+	NS[c]
D2 Δαgp18[b]	160–200	+	800–840
recp18 eluant[d]	60–100	+	2,400–3,000
gp18 eluant[d]	240–400	+	1,200–2,000
recp23 eluant[d]	NS	−	NS
recp40 eluant[d]	NS	−	NS
Ratαrecp18	320–480	+	>5,000
Mouseαgp18	160–320	+	>5,000

[a] IP of BDV and detection of genomic RNA by RT-PCR.

[b] Chronic rat sera (D2) adsorbed with recombinant (recp23, recp40, recp18) or native (gp18) protein.

[c] NS, not significant. The NT was considered to be not significant below 1:32; the recp18 ELISA titer was considered to be not significant below 1:250.

[d] Antibodies in chronic rat sera (D2) eluted from recombinant or native protein.

TABLE 5 Characterization of anti-gp18 MAbs

MAb	Ig class	Reciprocal NT	Characterization by: Western blot gp18	Western blot rp18	IP gp18	IP rp18	Reciprocal rp18 ELISA titer	IP-RT-PCR*
14/29A5	IgG2b	>400–1,000	+	+	+	+	500–1,000	–
14/26B9	IgM	8–16	–	–	+	+	4–8	+
14/8E1	IgM	50	–	–	+	+	200–300	+
14/13E10	IgM	50–100	–	–	+	+	16–64	+
14/18H7	IgG3	100–200	–	–	+	+	128–256	+

* IP of BDV and detection of genomic RNA by RT-PCR.

Monospecific antibodies to recp18 and gp18:

Sera from rats and mice immunized with recp18 and gp18, respectively, were tested for neutralization activity. Neutralizing antibodies in both rats and mice were detected at 12 weeks post-immunization. Sixteen-weeks after immunization with recp18, 2 rats had neutralization titers between 1:320 and 1:480 (Table 4); sera from 2 mice immunized with gp18 had neutralization titers between 1:160 and 1:320 (Table 4).

Monoclonal antibodies to gp18:

MAbs were generated against gp18. Five positive clones were identified by ELISA using gp18 as antigen. The MAbs represented three different immunoglobulin isotypes, yet all contained the kappa light chain (Table 5). Although each of the monoclonal antibodies immunoprecipitated gp18 (Table 5, FIG. 5A) and recp18 (Table 5), only MAb, 14/29A5 reacted by Western blot (Table 5, FIG. 15B).

Concentrated supernatants from all five MAbs neutralized BDV infectivity (Table 5). Similar to sera from chronically-infected rats (FIG. 16B), the neutralization titer of four MAbs was greatest at highest antibody concentration (FIG. 16C). In contrast, one MAb, 14/29A5, neutralized BDV only when used at a dilution of 1:400-1:1,000 (FIG. 16D). Neutralizing sera from chronically-infected rats, mice immunized with gp18 or rats immunized with recp18 (Table 4) had the capacity to inhibit 100% of BDV infectivity (FIG. 16B). In contrast, MAbs to gp18, used individually or in concert, inhibited a maximum of 68% of BDV infectivity (FIG. 16C and D). Supernatants of two MABS, 14/18H7, NT_{50} 1:16 and 14/13E10, NT_{50} 1:32, showed cooperativity in neutralization assays; pooling of these MAbs resulted in a higher neutralization titer (NT_{50} 1:100-150). To determine the extent to which neutralization was complement-dependent, neutralization activity of MAbs was tested with addition of either

active or heat-inactivated mouse complement. No increase in neutralization titer was detected with addition of mouse complement. Serum from noninfected (normal) rats was not neutralizing at dilutions greater than 1:16 (FIG. 16A).

Immunoprecipitation of BDV with neutralizing antibodies:

BDV stock was treated with nucleases to eliminate free nucleic acids then incubated with sera or MAbs and Protein A-Sepharose. RNA was extracted from immunoprecipitated viral particles or subparticles and subjected to RT-PCR for amplification of viral genomic RNA. Neutralizing rat sera (FIG. 17A), monospecific sera to recp18 (FIG. 17B) or gp18, and D2 serum antibodies eluted from recp18 or gp18 precipitated BDV particles. Removal of antibodies to recp23, recp40, recp18 or gp18 did not affect the capacity of neutralizing sera to precipitate viral particles. Four MAbs also precipitated BDV (FIG. 17B and Table 5). One MAb, 14/29A5, did not precipitate viral particles at any dilution (1:5, 1:100, 1:200 or 1:500). Sera from noninfected or two acutely infected rats (5 weeks p.i.) (FIG. 17A) did not precipitate BDV. Experiments with sera and monoclonal antibodies are summarized in Tables 4 and 5. Negative controls for RT-PCR included the omission of virus from immunoprecipitation (FIG. 17A) and the use of genomic sense primers for first strand cDNA synthesis (FIG. 17A).

DISCUSSION

The presence (or absence) of neutralizing antibodies in BDV-infected animals has been controversial. Some reports have not shown evidence for neutralizing antibodies {Carbone, K. M., et al., *J. Virol.*, 61:3431-3440 (1987); Herzog, S., et al., *J. Gen. Virol.*, 66:503-8 (1985) and Narayan, O., et al., *J. Inf. Dis.*, 148:305-315 (1983)}, however, this may reflect different timepoints for collection of sera or variation in the assay system for

neutralization. Although there are reports of neutralizing antibodies in serum and CSF of both naturally and experimentally infected animals {Danner, K., et al., *Zbl. Vet.-Med. [B]*, 25:345-355 (1978); Hirano, N., et al., *J. Gen. Virol.*, 64:1521-1530 (1983); Ludwig, H., et al., *Progr. Med. Virol.*, 35:107-151 (1988) and Ludwig, H., et al., *Arch. Virol. [Suppl]* 7:111-133 (1993)}, neither the timecourse for development of neutralizing antibodies nor their target antigens have been characterized. Here, we show that the neutralizing activity of BDV-rat sera increases dramatically from the acute (5 weeks p.i.) to the chronic (15 weeks p.i.) phase of disease and provide evidence to indicate that neutralization activity is due, at least in part, to antibodies that react with a BDV glycoprotein, gp18. The timecourse for the appearance of neutralizing antibodies seems to correlate with immunoreactivity to gp18. Furthermore, removal of antibodies to gp18 or recp18 dramatically decreased the neutralization titer of BDV-rat sera. In contrast, subtraction of antibodies to two other viral proteins, p40 and p23, had no effect.

Neutralization activity was detected with monospecific antiserum against both gp18 and recp18 as well as with monoclonal antibodies against gp18. These MAbs represent three different isotypes, IgM, IgG2b and IgG3, indicating that multiple isotypes are capable of virus neutralization. Addition of complement did not enhance neutralization activity of the MAbs, suggesting that the mechanism for neutralization was neither complement-mediated inactivation of virus nor steric hindrance by a complement-MAb-virus complex.

It is likely that at least three different antibody binding sites on gp18 were involved in neutralization. Four MAbs, which immunoprecipitated both gp18 and recp18 but did not detect protein in Western blots, presumably bound to discontinuous epitopes. The observation that use of MAbs 14/13E10 and 14/18H7 in combination, resulted

in greater neutralization activity than use of either MAb alone, suggests that these MAbs recognized either different discontinuous epitopes or different binding sites on a single discontinuous epitope. One MAb, 14/29A5, detected protein in Western blots as well as immunoprecipitation assays indicating that it bound to a continuous epitope. Unlike the other MAbs, 14/29A5 neutralized infectivity only after dilution (FIG. 16D), a profile consistent with neutralization by virus aggregation as reported in other viral systems {Dimmock, N. J., A. Capron, et al. (ed.), "Current Topics in Microbiology and Immunology", Springer-Verlag, Berlin (1993) and Outlaw, M. C., et al., *J. Gen. Virol.*, 71:69-76 (1990)}. Although all of the gp18 MAbs detected recp18 (nonglycosylated protein), it is possible that there are additional epitopes for neutralization which include the carbohydrate portion of gp18.

Sera from chronically-infected rats had greater neutralization activity than monospecific sera or monoclonal antibodies directed against gp18. Higher neutralization activity in sera from infected animals could reflect factors that influence epitope presentation such as interactions between gp18 and other proteins or the virion envelope. Alternatively, gp18 may not be the only BDV protein that elicits neutralizing antibodies. Sera from chronically-infected animals retained partial neutralizing activity and the capacity to precipitate virus after adsorption with gp18. Although this may be due to incomplete subtraction of antibodies to gp18 (Table 4) neutralizing antibodies may be directed against other viral proteins as well. For example, an additional candidate for a virion surface protein that may elicit neutralizing antibodies is p57. This putative protein contains multiple potential N-glycosylation sites and, as the product of the fourth ORF on the BDV genome, is in the gene position generally occupied by glycoproteins in *Mononegavirales* {Briese, T., et al., *Proc. Natl. Acad. Sci. USA*:

91:4362-4366 (1994)}. It is contemplated that passive administration of neutralizing antibodies or immunization with gp18 and other virion surface proteins can alter BDV pathogenesis.

EXAMPLE 5

Fragments of Borna Disease Virus Proteins Immunoactive With Sera From Human Schizophrenics and BDV Infected Animals

The etiology of schizophrenia, a debilitating disease that affects approximately 1% of the world's population, is unknown. Higher prevalence in some geographic areas, seasonal variation in births of subjects who develop disease, increased risk of schizophrenia in subjects exposed to influenza virus during the second trimester *in utero* and discordance for disease in monozygotic twins suggest the possibility of an infectious basis {Kirch, D. G., *Schizophrenia Bulletin*, 19:355-370 (1993)}. Borna disease virus has been implicated in human affective disorders by studies reporting that patients have serum antibodies to BDV {Rott, R., *et al.*, *Science*, 228:755-756 (1985)} and the presence of viral proteins and nucleic acids in peripheral blood mononuclear cells {Bode, L., *et al.*, *Nature Medicine*, 1:232-236 (1995)}. The catecholamine-related stereotypic behaviors observed in BDV-infected rats and catecholamine system dysfunction present in schizophrenia prompted Western blot (WB) studies of sera from schizophrenics for antibodies to BDV obtained from animals infected with the virus {Waltrip, R. W., II, *et al.*, *Psychiatry Res.*, 56:33-44 (1995)}. The present invention discloses an ELISA test for schizophrenia and BDV infection; fragments and peptides derived from p23 and gp18 which are immunoreactive with sera from schizophrenics and animals infected with BDV and/or immunized with p23 and gp18. The test is specific,

sensitive, fast, easy, and economical. For example, indirect immunofluorescence assays (IFT) used in the current art does not define the viral protein(s) responsible for immunoreactvity. IFT, immunoprecipitation (IP), and WB are also less sensitive than the ELISA of the present invention and require at least 2 days for completion and are unsuitable for screening of multiple samples. In contrast, the present ELISA provides inexpensive, rapid tests which allow analysis of hundreds of serum samples, *e.g.* in several hours, with minimal equipment. The advantages of ELISA over the prior art diagnostic methods are described in further detail in Briese, T., *et al.*, *J. Clin. Microbiol.*, 33:348-351 (1995). Besides the detection of schizophrenia and BDV infections disclosed in this Example, the ELISA method disclosed herein is generally applicable for studies and detection of neurologic and neuropsychiatric diseases and BDV infections in men and animals.

I. **Immunoreactivity of p40, p23 and gp18, with Sera from Schizophrenics.**

Sera from 30 human schizophrenic patients were examined by ELISA for immunoreactivity with recombinant BDV proteins N (recp40), P (recp23) and M (recp18) {The recombinant proteins were produced and the ELISA were performed as described in Examples 3 and 4, above, which were also described Briese, T., *et al.*, *J. Clin. Microbiol.*, 33:348-351 (1995)}. Controls were sera from 30 age and sex matched normal subjects and 30 patients with multiple sclerosis (MS), an autoimmune central nervous system (CNS) disease of unknown etiology. Although some sera detected N or P but not M, all sera immunoreactive with M also detected N and P. Twenty-seven percent of schizophrenic subjects (7) had serum antibodies to M, N and P versus 3% of normal subjects (1) or 0% of MS patients ($p < 0.0001$) (Table 6). Immunoreactivity of sera with all 3 proteins was confirmed by WB using extracts

from BDV-infected C6 cells supplemented with gp18; IP of the recombinant BDV proteins and IFT using infected rabbit fetal glial cells (the WB, IFT, and IP were conducted using the methods known in the art, as described in Briese, T., *et al.*, *J. Clin. Microbiol.*, 33:348-351 (1995)}). Sera not reactive with 1 or more of the 3 proteins in ELISA were also negative in the other assays.

TABLE 6

Percentage of subjects with schizophrenia (SZ), multiple sclerosis (MS) or no neuropsychiatric disease (NND) immunoreactive in ELISA with N, P and M proteins of

Borna disease virus

	N*	P†	M‡	N/P†	N/M‡	M/P†	N/P/M‡
SZ (n=30)	32	47	27	33	27	27	27
MS (n=30)	0	0	0	0	0	0	0
NND (n=30)	7	7	3	7	3	3	3

N = p40, P = p23, M = gp18

* $p < 0,01$; † $p < 0,001$; ‡ $p < 0.0001$

N/P = N and P

N/M = N and M

M/P = M and P

N/P/M = N, P and M

II. Selection and Immunoreactivity of Truncated Fragments of p23 and gp18

The immunologic determinants on p23 and gp18 were determined using truncated fragments of these proteins. The fragments used are shown in Figs. 20B and 21B. In FIG. 20B, the fragments are designated S1 to S4 and NS, respectively. In FIG. 21B, the fragments are derived from the unglycosylated version of recp18, and are denoted M1 to M4 and MS. The fragments are shown from the amino terminus (left) to the carboxyl terminus (right) of the proteins. The numbers below each fragment indicate the locations of the amino acids on the full length p23 or gp18, respectively. For example, p23 has a total of 201 amino acids, thus, as shown in FIG. 20B, S1 represents the full length p23 because it spans from amino acid at position 1 (denoted 1aa in the figure) to the amino acid at position 201 (denoted 201aa) of p23. S2 is a protein representing a fragment of p23, spanning from amino acid at position 37 to position 201 of p23. Similarly, in FIG. 21B, MS is a protein representing a fragment of the unglycosylated gp18, spanning from amino acid at position 1 to position 70 of the unglycosylated gp18. These fragments were recombinantly produced by selecting the appropriate PCR primers for the fragments based on the cDNA of p23 and gp18 disclosed in this patent application and by cloning the respective cDNA fragments into the prokaryotic expression vector pET15b (Novagen) using techniques known in the art such as described in Example 3, above.

To test their immunoreactivity, these truncated fragments of p23 and gp18 proteins were used in ELISA with sera from 6 BDV infected rats (15 weeks post infection) and 7 immunoreactive schizophrenic patients (of Section I above). Horse sera were only from acutely infected animals, at a stage of disease where antibodies to gp18 are not present, thus, sera from 4 BDV infected horses were used only to study p23. For truncated

fragments of p23, similar patterns of immunoreactivity were found for sera from BDV infected rats, BDV infected horses and schizophrenic patients. In the case of truncated fragments of unglycosylated gp18 proteins, instead of using sera from BDV infected horses, the sera from 2 mice immunized with native gp18 were tested (15 weeks post immunization). The immunized mice were used to determine whether the truncated fragments derived from unglycosylated gp18 were specifically immunoreactive with antibodies raised against native gp18. Again, similar patterns of immunoreactivity were found for sera from the BDV infected rats, mice immunized with native gp18, and schizophrenic patients. The above results are shown in Fig 20A and 21A, respectively, the taller blocks in the histograms indicate increased immunoreactivity relative to the shorter blocks. Significantly, the above truncated fragments did not immunoreact with sera from the same controls (and the same number of controls) used in Section I above, whereas some sera from human controls with no neuropsychiatric disease immunoreacted with the full length p23 and gp18 proteins (see Table 6). Thus, these fragments are more specific for detecting neuropsychiatric disease than the full length proteins.

III. **Epitope Mapping of Peptides Derived from p23 and gp18**

Fine-mapping of epitopes with overlapping peptides of p23 and gp18 also revealed that the same determinants were detected by the above sera. To determine where an epitope was within each protein, a series of overlapping peptides were chemically synthesized and each peptide was tested for its ability to bind the antibody from schizophrenics and the sera of animals infected with BDV and/or immunized with p23 and gp18.

As shown in Figs. 22 and 23, peptides of 8-mers were chemically synthesized, starting from the amino terminus of p23 and gp18 and spanning the full length of the

proteins. Except for the peptides at the amino and carboxyl termini of p23 and gp18, each of the intervening peptide overlaps its neighboring peptides (at its amino and carboxyl ends, respectively) by 4 amino acids.

To map the immunoepitopes on p23, the above 8-mer peptides derived from recp23 were tested against sera from: 6 rats infected with BDV (15 weeks post infection, p.I.); 2 rabbits immunized with recp23 (15 weeks post immunization); and 7 immunoreactive schizophrenic patients (of Section I above). To map the immunoepitopes on gp18, the above 8-mer peptides derived from unglycosylated recp18 were similarly tested, except that the rabbit sera were replaced with sera from 2 mice immunized with native gp18 (15 weeks post immunization). The immunized mice were used to determine whether the series of overlapping 8-mer peptides derived from unglycosylated gp18 were specifically immunoreactive with antibodies raised against native gp18. The controls in both tests were the same as in Section I above.

The tests were conducted using SPOTs membrane (Genosys Biotechnologies, Inc., The Woodlands, TX, USA) and the technique described in Frank, R., *et al.*, *Tetrahedron*, 44:6031-6040 (1988); Blankenmeyer-Menge, B., *et al.*, *Tetrahedron Letters*, 29(46):5871-5874 (1988); Blankenmeyer-Menge, B., *et al.*, in "Innovation and Perspectives in Solid-Phase Synthesis", (Epton, R. ed.), Chapman and Hall publ. (1989); and Blankenmeyer-Menge, B., *et al.*, *Tetrahedron Letters*, 32(12):1701-1704 (1990) (the tests are hereinafter described as the "SPOTs tests"). The results are shown in Figs. 22 and 23. The blocks in the histogram in each figure indicate the peptides which immunoreact with the sera, the taller block indicates increased immunoreactivity relative to the shorter blocks. Based on the immunoreactivity, the sequences of the epitopes were deduced. The amino acid sequences of the epitopes and thus the peptides are as shown in Tables 7 and 8,

below, wherein the peptide sequences are shown from the amino terminus (left) to the carboxyl terminus (right):

TABLE 7

Peptides derived from p23

MATRPSSL	SEQ ID No. 20
NALTQPVDQLLK	SEQ ID No. 21
DQPTGREQ	SEQ ID No. 22
VRGTLGDI	SEQ ID No. 23
TAQRCDHS	SEQ ID No. 24
METMKLMMEKVD	SEQ ID No. 25
PMLPSHPA	SEQ ID No. 26
TADEWDII	SEQ ID No. 27

TABLE 8

Peptides derived from gp18

MNSKHSYV	SEQ ID No. 28
TLMLEIDF	SEQ ID No. 29
GHSLVNIYFQID	SEQ ID No. 30
YKDPIRKY	SEQ ID No. 31
AFNVFSYR	SEQ ID No. 32

The result of representative SPOTs tests with the 8-mer peptides derived from unglycosylated gp18 are graphically shown in FIG. 24A, the panels contained sera from: 1 mouse immunized with native gp18, 1 rat infected with BDV, and 1 schizophrenic human, respectively. Each spot on the panels indicates the immunoreation of a serum sample with an 8-mer unglycosylated gp18 peptide. As shown in the scale on Fig 24B, the darker the spots, the higher the immunoreactivity. The lightest spot (Scale 1) indicates no detectable immunoreactivity; and the darkest spot (Scale 4) indicates highest immunoreactivity. As shown in FIG. 24A, the immunoreactivity pattern of the sera against the peptides were similar for all the animals/humans tested. Based on the pattern of

immunoreactivity as shown by the spots, the epitopes E1 to E5 were mapped. The result of the epitope mapping is graphically shown in FIG. 24B, the height of the blocks is directly proportional to the degree of immunoreactivity of the peptides tested which span the full length of gp18, from amino acid at position 1 to position 142. The sequences of the mapped epitopes, E1 to E5, are listed below the histogram of FIG. 24B. The epitopes mapped are the same as in Table 8, above, and confirmed that the sera were specifically immunoreactive with epitopes found within gp18. Again, significantly, the control sera did not immunoreact with the peptides.

The same test was applied to the overlapping 8-mer peptides derived from p23, except that the mice were immunized with recp23. A similar result was obtained, *i.e.* the immunoreactivity pattern of the sera against the peptides were similar for all the animals/humans tested, and the test produced the epitopes shown in Table 7, above. Again, significantly, the control sera did not immunoreact with the peptides.

In summary, the above truncated fragments, epitopes and peptides, and nucleotide sequences which encode them or which are complementary to these encoding nucleotide sequences, can be used to: (1) diagnose, prognose, monitor, and manage BDV infection/disease and schizophrenia, and more generally neurologic and neuropsychiatric diseases; and (2) vaccinate an animal or human against the foregoing infection and diseases. Other useful truncated immunoreactive fragments, epitopes and peptides can be similarly derived from the other BDV proteins using the method of this Example. Thus, the nucleotide sequences encoding these truncated fragments, epitopes and peptides, nucleotide sequences complementary to the foregoing, and recombinant vectors and cells expressing the truncated fragments, epitopes and peptides, and their uses are also claimed here. The vaccines, diagnostic, prognostic and monitoring methods,

recombinant vectors and cells, and nucleotide sequences, can be made using the teaching contained in this patent application in combination with methods known in the art. The above findings also suggest an association between BDV infection and schizophrenia.

EXAMPLE 6

Identification and Characterization of the BDV G-Protein

Although the BDV antigenome contains five major ORFs, products are reported only for the first three ORFs on the antigenome: N (p40), P (p24/p23) and M (gp18). The fourth ORF predicts a protein (G-protein) of 57 kDa that contains potential N-glycosylation sites. We have used a Semliki forest virus (SFV) vector to express the fourth ORF in BHK-21 cultured cells. The expressed protein migrated at 94 kDa in a 10% SDS-PAGE analysis. A 94 kDa BDV-specific protein was also identified in infected C6 cells by immunoprecipitation with sera from infected rats. The expressed protein was markedly sensitive to tunicamycin, endoglycosidase F/N-glycosidase and endoglycosidase H, indicating that the protein is an N-linked glycoprotein, largely comprised of high mannose- and/or hybrid-type oligosaccharides. SFVp57 transfected cells showed surface expression of the protein and formed syncitia. The protein's presence on the surface of transfected cells supports the hypothesis that the G-protein may be a virion surface attachment protein.

The fourth ORF in BDV predicts a protein of 57 kDa with N- and O- glycosylation sites and hydrophobic domains. Our findings show that in fact the fourth ORF encodes a BDV G-protein of approximately 94 kDa with multiple N-glycosylation and O-glycosylation sites and hydrophobic domains at the amino and carboxyl termini reminiscient of the signal sequence and transmembrane domains found in rhabdovirus G-proteins (FIG. 25). FIG.

25 shows the predicted amino acid sequence of the BDV G-protein (this BDV G-protein is also referred to as p57 in this patent application, and the amino acid sequence is listed as SEQ ID No. 8, above). Boxed regions represent the putative endoplasmic reticulum ("ER") signal peptide sequence (amino acids 7 to 20) and transmembrane domain (amino acids 468 to 488). Bold underlined sequences represent potential N-glycosylation sites. We now report that this ORF directs the expression of a 94-kDa N-linked glycoprotein.

Expression of a 94 kDa protein from the BDV p57 ORF using an SFV expression vector. The p57 ORF (nt. 2229 to nt. 3744; Strain V) {Briese, T., *et al.*, *Proc. Natl. Acad. Sci. USA*, 91:4362-4366 (1994)} was amplified by RT-PCR from BDV-infected C6 cell RNA by using primers 5'-CGCAATCAATGCAGC (SEQ ID NO 34) and 5'-TTCCTGCCACCGGCCG (SEQ ID NO 35) and cloned into the SmaI restriction site in vector pSFV-1 (GibcoBRL, Life Technologies, Inc., Grand Island, New York) {Marchuk, D., *et al.*, *Nucleic Acids Res.*, 19:1154 (1990)} to create pSFVp57. Capped SFV genomic RNA encoding p57 or β-galactosidase (control), were transcribed from pSFVp57 or pSFV3LacZ template, respectively. After tranfection into BHK-21 cells, the over expressed proteins were analyzed by immunohistochemical and biochemical assays {Kriegler, M., *In Gene transfer and expression: a laboratory manual*, p. 219-224, Stockton Press, New York (1990)}. The SFVp57 cells formed large multinucleated syncytia and expressed a surface protein detected by sera from BDV infected rats ("BD-rat sera" or "BDSe") but not normal rat sera (NLSe). SFVLacZ cells did not form syncytia, or express proteins reactive with either BDSe or NLSe. The syncytia formation and surface protein expression observed in cells transfected with SFV-p57 were similar to those described in cells transfected with SFV vectors containing other glycoproteins {Gallaher, W. R., *et al.*, *J.*

Virol., 14:813-820 (1974); Paul, N. L., *et al.*, *AIDS Res. and Hum. Retroviruses*, 9:963-970 (1993)}.

Lysates of metabolically-labeled SFVp57 and SFVLacZ cells were used for immunoprecipitation (IP) experiments with BDSe and NLSe. Approximately 2 X 10^4 transfected cells (16 hrs after electroporation) were incubated for two hours in 1 ml of methionine-minus Modified Eagles Medium (GibcoBRL, Life Technologies, Inc., Grand Island, New York). Thereafter, 0.2mCi of ^{35}[S]Met-Cys-protein label mix (New England Nuclear, Boston, Massachusetts) was added for eight hours to radiolabel newly synthesized proteins. Cell lysates were subjected to IP according to Yamashita *et al.* {Yamashita, Y., *et al.*, *J. Virol.*, 68:7933-7943 (1994)} except for the modification that protein G-sepharose (Sigma) was substituted for protein A. After SDS-PAGE and autoradiography, a 94 kDa protein was detected in lysates of SFVp57 cells but not in lysates of SFVLacZ cells.

Identification of a BDV-specific 94 kDa protein in BDV-infected C6 cells. Lysates of BDV-infected C6 cells (C6BDV) and non-infected C6 cells were metabolically-labeled and IP with BDSe or NLSe for analysis by SDS-PAGE and autoradiography (see above). At least six BDV-specific proteins were detected in lysates from infected cells by BDSe that were not detected in lysates of infected cells by NLSe or in lysates of noninfected cells by BDSe. These included proteins of 200 kDa (pol), 94 kDa (G-protein), 40 kDa (N protein), 36 kDa, 33 kDa and 23 kDa (P protein). Whether the 36 kDa or 33 kDa proteins are of viral or host origin is unknown.

Characterization of the BDV 94 kDa protein. Metabolically-labeled SFV-p57 and SFVLacZ cells were treated with 10 μg/ml tunicamycin to inhibit N-linked glycosylation. Cell lysates were used in IP experiments with BDSe or NLSe prior to SDS-PAGE and autoradiography.

BDSe immunoprecipitated proteins of 94 kDa and 64 kDa from SFVp57 cell lysates. The 94 kDa protein was detected in untreated cells but not in tunicamycin-treated cells. Conversely, the 64 kDa protein was detected in treated cells but not in untreated cells. Neither protein was detected in SFVLacZ cells.

Radiolabeled proteins immunoprecipitated by BDSe were eluted from the sepharose beads by incubation in sixty microliters of 50 mMTris-HCl, pH 6.8, 0.4% SDS, 0.1M 2-mercaptoethanol at 95°C for 10 min and digested with endoglycosidase H (Endo H) (Boehringer Mannheim); endoglycosidase F and N-glycosidase F (Endo F/PNGase F) (J. Elder); Endo H, neuraminidase (Boehringer Mannheim) and O-glycosidase (Boehringer Mannheim); or neuraminidase and O-glycosidase. Methods for carbohydrate digestion followed protocols of the manufacturer (Endo H, neuraminidase, O-glycosidase) or Alexander and Elder (Endo F/PNGase F) {Alexander, S., *et al.*, *Meth. Enzymol.*, 179:505-518 (1989)}; using Endo H, 2.0 mU; Endo F/PNGase F, 25 mU; O-glycosidase, 0.8 mU; neuraminidase, 1.0 mU in 40 microliter reactions. Controls for these reactions included incubation of proteins with digestion buffer alone. Radiolabeled p57 was prepared by *in vitro* translation in rabbit reticulocytes in the absence of microsomal membranes to provide a nonglycosylated G-protein standard {Lipkin, W. I., *et al.*, *Proc. Natl. Acad. Sci. USA*, 87:4184-4188 (1990)}. Proteins were subjected to 10% SDS-PAGE and analyzed by autoradiography. Treatment with endo H or endo F/PNGase F resulted in an apparent shift in approximately mw from 94 kDa to 64 kDa, the position of radiolabeled nonglycosylated G-protein. Treatment with neuraminidase and O-glycosidase did not allow resolution of a shift. Therefore, to enhance the sensitivity of SDS-PAGE for identifying small shifts in mw following O-glycosidase digestion, protein was first incubated with endo H. Comparison of protein digested with endo H alone with protein digested with endo H,

neuraminidase and O-glycosidase revealed a subtle shift of less than 1 kDa.

Antibodies to the BDV 94 kDa protein in BDV-infected rats. Viral glycoproteins tend to be immunoreactive and are often targets for neutralizing antibodies. Previous studies of the BDV M-protein revealed epitopes that bind neutralizing antibodies {Hatalski, C. G., *et al.*, *J. Virol.*, 69:741-747 (1995)}. Because adsorption experiments using purified M-protein did not completely abrogate neutralization activity in chronic BDSe, it was proposed that additional neutralization epitopes might be present on the putative G-protein {Hatalski, C. G., *et al.*, *J. Virol.*, 69:741-747 (1995)}. To address the possibility that serum antibodies to G-protein might be present at higher titer in chronic BD-rat, sera from BD-rats sacrificed at different phases of disease were used to IP proteins from metabolically-labeled SFVp57 cells for analysis by SDS-PAGE and autoradiography. The 94 kDa protein signal was approximately 10-fold higher after IP with sera collected from animals 3 months post infection (chronic phase) than after IP with sera collected from animals 1 month post infection (acute phase).

This study was initiated to identify and characterize the product of the fourth ORF on the BDV antigenome. Expression of this ORF in SFVp57 cells yielded a 94 kDa glycoprotein. Inhibition of carbohydrate conjugation by tunicamycin and endo F/PNGase F sensitivity indicate a primary role for N-linkage. Sensitivity to endo H suggests that the N-linked carbohydrate is largely comprised of high mannose- and/or hybrid-type oligosaccharides {Tarentino, A. L., *et al.*, *Methods Enzymol.*, 50:574-580 (1979)}. The subtle shift in apparent mw after digestion with neuraminidase and O-glycosidase may represent the presence of O-linked

carbohydrate {Hawkins, L. K., *et al.*, *Virol.*, **210**:335-344 (1995)}.

Although there are no direct data to indicate a function for BDV G-protein, several observations suggest that it could play a role in viral attachment and/or penetration. First, the BDV G-protein contains a carboxyl transmembrane domain and localizes to the plasma membrane in pSFV57 cells. These features are reminiscient of other enveloped viral systems where G-proteins mediate early events in infection {White, J., *et al.*, *Q. Rev. Biol. Phys.*, **16**:151-195 (1983)}. Second, treatment of BDV particle preparations with N-acetylglucosaminidase and mannosidase reduced infectivity suggesting the importance of N-acetylglucosamine and mannose residues in terminal positions {Stoyloff, R., *et al.*, *Arch. Virol.*, **137**:405-409 (1994)}. Although the BDV M-protein may serve as a viral attachment protein, it does not contain terminal mannose residues {Kliche, S., *et al.*, *J. Virol.*, **68**:6918-6923 (1994)}. In contrast, the BDV G-protein does include terminal mannose residues and could therefore represent the sensitive virion surface component identified in particle infectivity experiments. Third, the titer of antibodies to G-protein in infected rats increased dramatically in the chronic phase of infection, consistent with the timecourse for appearance of neutralizing antibodies {Hatalski, C. G., *et al.*, *J. Virol.*, **69**:741-747 (1995)}.

All publications and patent applications mentioned in this Specification are herein incorporated by reference to the same extent as if each of them had been individually indicated to be incorporated by reference.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity and understanding, it will be obvious that various modifications and changes which are within

the skill of those skilled in the art are considered to fall within the scope of the appended claims. Future technological advancements which allows for obvious changes in the basic invention herein are also within the claims.

Deposit

The cDNA of BDV genomic RNA sequence has been deposited in the GenBank data base (accession no. U04608). This GenBank sequence is hereby incorporated by reference in its entirety.

The recombinant transfer vector, suitable for transformation into *Escherichia coli* DH10, containing four overlapping cDNA libraries (as described in Example 1, above) representing the entire BDV viral genome has been deposited under the Budapest Treaty, at the American Type Culture Collection, Rockville, MD 20852 (U.S.A.) on December 30, 1994 under the deposit name BDVU04608, and ATCC Accession No. 97008.

Availability of the deposited recombinant tranfer vector is not to be construed as a license to practice the invention in contravention of the rights granted under the authority of any government in accordance with its patent laws.

Also, the present invention is not to be considered limited in scope by the deposited recombinant transfer vector, since the deposited vector is intended only to be illustrative of particular aspects of the invention. Any recombinant transfer vector which can be used to prepare recombinant microorganism which can function to produce a recombinant protein product described herein is considered to be within the scope of this invention. Further, various modifications of the invention in addition to those shown and described herein which are apparent to those skilled in the art from the preceding description are considered to fall within the scope of the appended claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: The Regents of the University of California

(ii) TITLE OF INVENTION: Borna Disease Viral Sequences, Diagnostics and Therapeutics for Central Nervous System Diseases

(iii) NUMBER OF SEQUENCES: 35

(iv) CORRESPONDENCE ADDRESS:
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(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/369,822
APPLICATION NUMBER: US 08/434,831
APPLICATION NUMBER: Unknown
(B) FILING DATE: 06-JAN-1995
FILING DATE: 04-MAY-1995
FILING DATE: 04-JANUARY-1996
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Berliner, Robert
(B) REGISTRATION NUMBER: 20,121
(C) REFERENCE/DOCKET NUMBER: 5555-357C2

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 213/977-1001
(B) TELEFAX: 213/977-1003

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1112 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

ATGCCACCCA AGAGACGCCT GGTTGATGAC GCCGATGCCA TGGAGGATCA AGATCTATAT 60

GAACCCCCAG CGAGCCTCCC TAAGCTCCCT GGGAAATTCC TACAATACAC CGTTGGGGGG 120

TCTGACCCGC ATCCGGGTAT AGGGCATGAG AAAGACATCA GGCAGAACGC AGTGGCATTG 180

TTAGACCAGT CACGGCGCGA TATGTTTCAC ACAGTAACGC CTAGCCTTGT GTTTCTATGT 240

TTGCTAATCC CAGGACTGCA CGCTGCGTTT GTTCACGGAG GGGTGCCTCG TGAATCCTAC 300

CTGTCGACGC CTGTCACGCG TGGAGAACAG ACTGTTGTTA AGACTGCGAA GTTTTACGGG 360

GAAAAGACGA CGCAGCGTGA TCTCACCGAG CTGGAGATCT CCTCTATCTT CAGCCATTGT 420

TGCTCATTAC TAATAGGGGT TGTGATAGGA TCGTCGTCTA AGATCAAAGC AGGAGCCGAG 480

CAGATCAAGA AAAGGTTTAA AACTATGATG GCAGCCTTAA ACCGGCCATC CCATGGTGAG 540

ACTGCTACAC TACTCCAGAT GTTTAATCCA CATGAGGCTA TAGATTGGAT TAACGGCCAA 600

CCCTGGGTAG GCTCCTTTGT GTTGTCTCTA CTAACTACAG ACTTTGAGTC CCCAGGTAAA 660

GAATTTATGG ACCAGATTAA GCTTGTCGCA AGTTATGCAC AGATGACTAC GTACACTACT 720

ATAAAGGAGT ACCTCGCAGA ATGCATGGAT GCTACCCTTA CAATCCCCGT AGTTGCATAT 780

GAGATCCGTG ACTTTTTAGA AGTTTCAGCA AAGCTTAAGG AGGATCATGC TGACCTGTTC 840

CCGTTTCTGG GGGCCATTAG ACACCCCGAC GCTATCAAGC TGGCGCCACG AAGCTTTCCC 900

AATCTGGCCT CCGCAGCGTT TTACTGGAGT AAGAAGGAAA ACCCCACAAT GGCAGGCTAC 960

CGGGCCTCCA CCATCCAGCC GGGCGCAAGT GTCAAGGAAA CCCAGCTTGC CCGGTATAGG 1020

CGCCGCGAGA TATCTCGTGG AGAGGACGGG GCAGAGCTCT CAGGTGAGAT CTCTGCCATA 1080

ATGAAGATGA TAGGTGTGAC TGGTCTAAAC TA 1112

(3) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 370 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Pro Pro Lys Arg Arg Leu Val Asp Asp Ala Asp Ala Met Glu Asp
1 5 10 15

Gln Asp Leu Tyr Glu Pro Pro Ala Ser Leu Pro Lys Leu Pro Gly Lys
20 25 30

Phe Leu Gln Tyr Thr Val Gly Gly Ser Asp Pro His Pro Gly Ile Gly
35 40 45

His Glu Lys Asp Ile Arg Gln Asn Ala Val Ala Leu Leu Asp Gln Ser
50 55 60

Arg Arg Asp Met Phe His Thr Val Thr Pro Ser Leu Val Phe Leu Cys
65 70 75 80

Leu Leu Ile Pro Gly Leu His Ala Ala Phe Val His Gly Gly Val Pro
85 90 95

Arg Glu Ser Tyr Leu Ser Thr Pro Val Thr Arg Gly Glu Gln Thr Val
100 105 110

Val Lys Thr Ala Lys Phe Tyr Gly Glu Lys Thr Thr Gln Arg Asp Leu
115 120 125

Thr Glu Leu Glu Ile Ser Ser Ile Phe Ser His Cys Cys Ser Leu Leu

130 135 140

Ile Gly Val Val Ile Gly Ser Ser Ser Lys Ile Lys Ala Gly Ala Glu
145 150 155 160

Gln Ile Lys Lys Arg Phe Lys Thr Met Met Ala Ala Leu Asn Arg Pro
165 170 175

Ser His Gly Glu Thr Ala Thr Leu Leu Gln Met Phe Asn Pro His Glu
180 185 190

Ala Ile Asp Trp Ile Asn Gly Gln Pro Trp Val Gly Ser Phe Val Leu
195 200 205

Ser Leu Leu Thr Thr Asp Phe Glu Ser Pro Gly Lys Glu Phe Met Asp
210 215 220

Gln Ile Lys Leu Val Ala Ser Tyr Ala Gln Met Thr Thr Tyr Thr Thr
225 230 235 240

Ile Lys Glu Tyr Leu Ala Glu Cys Met Asp Ala Thr Leu Thr Ile Pro
245 250 255

Val Val Ala Tyr Glu Ile Arg Asp Phe Leu Glu Val Ser Ala Lys Leu
260 265 270

Lys Glu Asp His Ala Asp Leu Phe Pro Phe Leu Gly Ala Ile Arg His
275 280 285

Pro Asp Ala Ile Lys Leu Ala Pro Arg Ser Phe Pro Asn Leu Ala Ser
290 295 300

Ala Ala Phe Tyr Trp Ser Lys Lys Glu Asn Pro Thr Met Ala Gly Tyr
305 310 315 320

Arg Ala Ser Thr Ile Gln Pro Gly Ala Ser Val Lys Glu Thr Gln Leu
325 330 335

Ala Arg Tyr Arg Arg Arg Glu Ile Ser Arg Gly Glu Asp Gly Ala Glu
340 345 350

Leu Ser Gly Glu Ile Ser Ala Ile Met Lys Met Ile Gly Val Thr Gly
355 360 365

Leu Asn
370

(4) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 609 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

ATGGCAACGC GACCATCGAG TCTGGTCGAC TCCCTGGAGG ACGAAGAAGA TCCCCAGACA 60

CTACGACGGG AACGACCGGG GTCACCAAGA CCACGGAAGG TCCCAAGGAA TGCATTGACC 120

CAACCAGTAG ACCAGCTCCT GAAGGACCTC AGGAAGAACC CCTCCATGAT CTCAGACCCA 180

GACCAGCGAA CCGGAAGGGA GCAGCTGTCG AATGATGAGC TAATCAAGAA GTTAGTGACG 240

GAGCTGGCCG AGAATAGCAT GATCGAGGCT GAGGAGGTGC GGGGCACTCT TGGAGACATC 300

TCGGCTCGTA TCGAGGCAGG GTTTGAGTCC CTGTCCGCCC TCCAAGTGGA AACCATCCAG 360

ACAGCTCAGC GGTGCGATCA CTCCGACAGC ATCAGGATCC TCGGCGAGAA CATCAAGATA 420

CTAGATCGCT CCATGAAGAC AATGATGGAG ACAATGAAGC TCATGATGGA GAAGGTGGAT 480

CTCCTCTACG CATCAACCGC CGTTGGGACC TCTGCACCCA TGTTGCCCTC CCATCCTGCA 540

CCTCCGCGCA TTTATCCCCA GCTCCCAAGT GCCCCGACAA CGGATGAATG GGACATCATA 600

CCATAAAAA 609

(5) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 201 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Ala Thr Arg Pro Ser Ser Leu Val Asp Ser Leu Glu Asp Glu Glu
1 5 10 15

Asp Pro Gln Thr Leu Arg Arg Glu Arg Pro Gly Ser Pro Arg Pro Arg
20 25 30

Lys Val Pro Arg Asn Ala Leu Thr Gln Pro Val Asp Gln Leu Leu Lys
35 40 45

Asp Leu Arg Lys Asn Pro Ser Met Ile Ser Asp Pro Asp Gln Arg Thr
50 55 60

Gly Arg Glu Gln Leu Ser Asn Asp Glu Leu Ile Lys Lys Leu Val Thr
65 70 75 80

Glu Leu Ala Glu Asn Ser Met Ile Glu Ala Glu Glu Val Arg Gly Thr
85 90 95

Leu Gly Asp Ile Ser Ala Arg Ile Glu Ala Gly Phe Glu Ser Leu Ser
100 105 110

Ala Leu Gln Val Glu Thr Ile Gln Thr Ala Gln Arg Cys Asp His Ser
115 120 125

Asp Ser Ile Arg Ile Leu Gly Glu Asn Ile Lys Ile Leu Asp Arg Ser
130 135 140

Met Lys Thr Met Met Glu Thr Met Lys Leu Met Met Glu Lys Val Asp
145 150 155 160

Leu Leu Tyr Ala Ser Thr Ala Val Gly Thr Ser Ala Pro Met Leu Pro
165 170 175

Ser His Pro Ala Pro Pro Arg Ile Tyr Pro Gln Leu Pro Ser Ala Pro
180 185 190

Thr Thr Asp Glu Trp Asp Ile Ile Pro
195 200

(6) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 428 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

ATGAATTCAA AACATTCCTA TGTGGAGCTC AAGGACAAGG TAATCGTCCC TGGATGGCCC 60

ACACTGATGC TTGAGATAGA CTTTGTAGGG GGGACTTCAC GGAACCAGTT CCTTAACATC 120

CCATTTCTTT CAGTGAAAGA GCCTCTGCAG CTTCCACGCG AGAAGAAGTT GACCGACTAC 180

TTTACTATTG ACGTAGAACC AGCAGGTCAT TCCCTGGTCA ATATATACTT CCAGATTGAC 240

GACTTCTTGC TCCTAACACT CAACTCACTA TCTGTGTACA AGGACCCGAT TAGAAAATAC 300

ATGTTCCTAC GCCTCAACAA GGACCAGAGC AAGCACGCAA TCAATGCAGC CTTCAATGTC 360

TTTTCTTATC GGCTTCGGAA CATTGGTGTT GGTCCTCTCG GCCCGGACAT TCGATCTTCA 420

GGGCCTTA 428

(7) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 142 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met Asn Ser Lys His Ser Tyr Val Glu Leu Lys Asp Lys Val Ile Val
1 5 10 15

Pro Gly Trp Pro Thr Leu Met Leu Glu Ile Asp Phe Val Gly Gly Thr
20 25 30

Ser Arg Asn Gln Phe Leu Asn Ile Pro Phe Leu Ser Val Lys Glu Pro
35 40 45

Leu Gln Leu Pro Arg Glu Lys Lys Leu Thr Asp Tyr Phe Thr Ile Asp
50 55 60

Val Glu Pro Ala Gly His Ser Leu Val Asn Ile Tyr Phe Gln Ile Asp
65 70 75 80

Asp Phe Leu Leu Leu Thr Leu Asn Ser Leu Ser Val Tyr Lys Asp Pro
85 90 95

Ile Arg Lys Tyr Met Phe Leu Arg Leu Asn Lys Asp Gln Ser Lys His
100 105 110

Ala Ile Asn Ala Ala Phe Asn Val Phe Ser Tyr Arg Leu Arg Asn Ile
115 120 125

Gly Val Gly Pro Leu Gly Pro Asp Ile Arg Ser Ser Gly Pro
130 135 140

(8) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1515 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

ATGCAGCCTT CAATGTCTTT TCTTATCGGC TTCGGAACAT TGGTGTTGGT CCTCTCGGCC 60

CGGACATTCG ATCTTCAGGG CCTTAGCTGC AATACTGACT CCACTCCTGG ACTGATTGAC 120

CTGGAGATAA GGCGACTTTG CCACACCCCA ACGGAAAATG TCATTTCATG CGAGGTTAGT 180

TATCTCAACC ACACGACTAT TAGCCTCCCG GCAGTCCACA CATCATGCCT CAAGTACCAC 240

TGCAAAACCT ATTGGGGATT CTTTGGTAGC TACAGCGCTG ACCGAATCAT AAATCGGTAC 300

ACTGGTACTG TTAAGGGTTG TCTAAACAAC TCAGCACCAG AGGACCCCTT CGAGTGCAAC 360

TGGTTCTACT GCTGCTCGGC GATTACAACA GAGATCTGCC GATGCTCTAT TACAAATGTC 420

ACGGTGGCTG TGCAAACATT CCCACCGTTC ATGTACTGCA GTTTTGCAGA CTGCAGTACC 480

GTGAGCCAAC AGGAGCTAGA GAGTGGAAAG GCAATGCTGA GCGATGGCAG TACATTAACT 540

TATACCCCGT ATATCCTACA GTCAGAAGTC GTGAACAAAA CCCTCAATGG GACCATACTC 600

TGCAACTCAT CCTCTAAGAT AGTTTCCTTC GATGAATTTA GGCGTTCATA CTCCCTAACG 660

AATGGTAGTT ACCAGAGCTC ATCAATCAAT GTGACGTGTG CAAACTACAC GTCGTCCTGC 720

CGGCCCAGGT TGAAAAGGCG GCGTAGGGAC ACCCAGCAGA TTGAGTATCT AGTTCACAAG 780

CTTAGGCCCA CACTGAAAGA TGCATGGGAG GACTGTGAGA TCCTCCAGTC TCTGCTCCTA 840

GGGGTGTTTG GTACTGGGAT CGCAAGTGCT TCTCAATTTT TGAGGAGCTG GCTCAACCAC 900

CCTGACATCA TCGGGTATAT AGTTAATGGA GTTGGGGTTG TCTGGCAATG CCATCGTGTT 960

AATGTCACGT TCATGGCGTG GAATGAGTCC ACCTATTACC CTCCAGTAGA TTACAATGGG 1020

CGGAAGTACT TCCTGAATGA TGAGGGAAGG TTACAAACAA ACACCCCCGA GGCAAGGCCA 1080

GGGCTTAAGC GGGTCATGTG GTTCGGCAGG TACTTCCTAG GGACAGTAGG GTCTGGGGTG 1140

AAACCGAGGA GGATTCGGTA CAATAAGACC TCACATGACT ACCACCTGGA GGAGTTTGAG 1200

GCAAGTCTCA ACATGACCCC TCAGACCAGT ATCGCCTCGG GTCATGAGAC AGACCCCATA 1260

AATCATGCCT ACGGAACGCA GGCTGATCTC CTTCCATACA CCAGGTCTAG TAATATAACA 1320

TCTACGGATA CAGGCTCAGG CTGGGTGCAC ATCGGCCTAC CCTCATTTGC TTTCCTCAAT 1380

CCCCTCGGGT GGCTCAGGGA CCTACTTGCA TGGGCAGCCT GGTTGGGTGG GGTTCTATAC 1440

TTAATAAGTC TTTGTGTTTC CTTACCAGCC TCCTTCGCGA GGAGGAGACG CCTCGGCCGG 1500

TGGCAGGAAT AAACC 1515

(9) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 503 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```
Met Gln Pro Ser Met Ser Phe Leu Ile Gly Phe Gly Thr Leu Val Leu
1               5                  10                  15

Val Leu Ser Ala Arg Thr Phe Asp Leu Gln Gly Leu Ser Cys Asn Thr
            20                  25                  30

Asp Ser Thr Pro Gly Leu Ile Asp Leu Glu Ile Arg Arg Leu Cys His
        35                  40                  45

Thr Pro Thr Glu Asn Val Ile Ser Cys Glu Val Ser Tyr Leu Asn His
    50                  55                  60

Thr Thr Ile Ser Leu Pro Ala Val His Thr Ser Cys Leu Lys Tyr His
65                  70                  75                  80

Cys Lys Thr Tyr Trp Gly Phe Phe Gly Ser Tyr Ser Ala Asp Arg Ile
                85                  90                  95

Ile Asn Arg Tyr Thr Gly Thr Val Lys Gly Cys Leu Asn Asn Ser Ala
            100                 105                 110

Pro Glu Asp Pro Phe Glu Cys Asn Trp Phe Tyr Cys Cys Ser Ala Ile
        115                 120                 125

Thr Thr Glu Ile Cys Arg Cys Ser Ile Thr Asn Val Thr Val Ala Val
    130                 135                 140

Gln Thr Phe Pro Pro Phe Met Tyr Cys Ser Phe Ala Asp Cys Ser Thr
145                 150                 155                 160

Val Ser Gln Gln Glu Leu Glu Ser Gly Lys Ala Met Leu Ser Asp Gly
                165                 170                 175

Ser Thr Leu Thr Tyr Thr Pro Tyr Ile Leu Gln Ser Glu Val Val Asn
            180                 185                 190

Lys Thr Leu Asn Gly Thr Ile Leu Cys Asn Ser Ser Ser Lys Ile Val
        195                 200                 205

Ser Phe Asp Glu Phe Arg Arg Ser Tyr Ser Leu Thr Asn Gly Ser Tyr
    210                 215                 220

Gln Ser Ser Ser Ile Asn Val Thr Cys Ala Asn Tyr Thr Ser Ser Cys
225                 230                 235                 240

Arg Pro Arg Leu Lys Arg Arg Arg Arg Asp Thr Gln Gln Ile Glu Tyr
                245                 250                 255

Leu Val His Lys Leu Arg Pro Thr Leu Lys Asp Ala Trp Glu Asp Cys
            260                 265                 270

Glu Ile Leu Gln Ser Leu Leu Leu Gly Val Phe Gly Thr Gly Ile Ala
        275                 280                 285

Ser Ala Ser Gln Phe Leu Arg Ser Trp Leu Asn His Pro Asp Ile Ile
    290                 295                 300

Gly Tyr Ile Val Asn Gly Val Gly Val Val Trp Gln Cys His Arg Val
305                 310                 315                 320

Asn Val Thr Phe Met Ala Trp Asn Glu Ser Thr Tyr Tyr Pro Pro Val
                325                 330                 335

Asp Tyr Asn Gly Arg Lys Tyr Phe Leu Asn Asp Glu Gly Arg Leu Gln
            340                 345                 350

Thr Asn Thr Pro Glu Ala Arg Pro Gly Leu Lys Arg Val Met Trp Phe
        355                 360                 365

Gly Arg Tyr Phe Leu Gly Thr Val Gly Ser Gly Val Lys Pro Arg Arg
    370                 375                 380
```

Ile Arg Tyr Asn Lys Thr Ser His Asp Tyr His Leu Glu Glu Phe Glu
385 390 395 400

Ala Ser Leu Asn Met Thr Pro Gln Thr Ser Ile Ala Ser Gly His Glu
405 410 415

Thr Asp Pro Ile Asn His Ala Tyr Gly Thr Gln Ala Asp Leu Leu Pro
420 425 430

Tyr Thr Arg Ser Ser Asn Ile Thr Ser Thr Asp Thr Gly Ser Gly Trp
435 440 445

Val His Ile Gly Leu Pro Ser Phe Ala Phe Leu Asn Pro Leu Gly Trp
450 455 460

Leu Arg Asp Leu Leu Ala Trp Ala Ala Trp Leu Gly Gly Val Leu Tyr
465 470 475 480

Leu Ile Ser Leu Cys Val Ser Leu Pro Ala Ser Phe Ala Arg Arg Arg
485 490 495

Arg Leu Gly Arg Trp Gln Glu
500

(10) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5135 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

ATGTCATTTC ATGCGAGCCT CCTTCGCGAG GAGGAGACGC CTCGGCCGGT GGCAGGAATA 60

AACCGTACCG ACCAGTCTCT TAAAAACCCT CTCCTCGGAA CAGAGGTCTC TTTCTGCCTT 120

AAGTCGAGCT CACTCCCCCA TCATGTACGA GCACTAGGCC AGATTAAAGC AAGGAACCTG 180

GCATCCTGTG ACTATTACTT GCTATTCCGC CAAGTTGTAT TGCCCCCTGA AGTATATCCC 240

ATTGGTGTTC TAATAAGAGC TGCGGAGGCT ATACTAACAG TTATAGTATC AGCTTGGAAG 300

CTGGATCATA TGACGAAGAC CCTATACTCC TCTGTGAGAT ATGCACTCAC CAATCCCCGG 360

GTCCGAGCCC AACTTGAGCT TCACATTGCC TACCAGCGCA TAGTGGGTCA GGTCTCGTAC 420

AGCCGGGAGG CAGACATAGG GCCAAAAAGG CTTGGGAATA TGTCATTGCA ATTCATCCAA 480

TCTCTCGTTA TTGCCACCAT AGACACGACA AGCTGCCTAA TGACCTACAA CCACTTTCTT 540

GCTGCAGCAG ACACAGCCAA GAGCAGATGC CATCTCCTAA TCGCCTCAGT GGTCCAGGGG 600

GCCCTTTGGG AACAAGGGTC ATTTCTTGAT CATATAATCA ACATGATCGA CATAATTGAC 660

TCAATCAACC TCCCCCATGA TGATTACTTC ACAATTATTA AGTCTATCTT TCCCTACTCC 720

CAAGGGCTTG TTATGGGGAG GCATAATGTA TCAGTCTCCT CTGATTTCGC GTCCGTATTT 780

GCCATTCCTG AATTATGCCC GCAACTAGAC AGCTTACTAA AAAAACTGCT CCAACTTGAC 840

CCCGTTCTCC TCCTCATGGT CTCTTCGGTG CAGAAGTCAT GGTACTTCCC TGAGATCCGA 900

ATGGTCGACG GGTCACGGGA GCAGCTCCAC AAGATGCGTG TCGAGCTGGA AACGCCCCAA 960

GCCCTGCTGT CGTACGGCCA TACCCTCCTG TCAATATTTC GGGCAGAGTT TATCAAAGGC 1020

TATGTCTCAA AGAATGCGAA GTGGCCGCCC GTACACCTGC TCCCAGGCTG TGACAAATCC 1080

ATAAAAAATG CGAGAGAGCT GGGCCGCTGG AGCCCGGCAT TTGACCGACG ATGGCAGCTC 1140

TTCGAGAAGG TTGTCATTCT AAGAATTGCT GACCTAGATA TGGATCCCGA CTTCAACGAT 1200

ATTGTTAGCG ATAAGGCGAT AATCAGCTCA AGAAGGGACT GGGTATTCGA GTACAATGCA 1260

GCGGCCTTTT GGAAGAAATA CGGTGAACGG TTGGAGAGGC CTCCTGCCAG GTCGGGACCG 1320

TCACGACTTG TGAATGCTCT AATCGATGGA CGCTTAGACA ATATCCCAGC CCTGCTAGAG 1380

CCATTTTACA GGGGAGCGGT TGAGTTCGAG GATCGGTTGA CTGTGCTCGT GCCTAAGGAG 1440

AAAGAGTTAA AGGTAAAGGG AAGGTTCTTC TCGAAGCAAA CATTGGCAAT CAGGATATAT 1500

CAGGTTGTTG CTGAAGCTGC ACTTAAGAAT GAGGTTATGC CATACCTAAA GACACACTCA 1560

ATGACCATGA GCTCAACGGC TCTAACTCAC CTTCTTAACC GGCTATCACA TACTATCACT 1620

AAGGGTGACT CCTTTGTTAT TAACCTTGAC TATAGTTCCT GGTGCAACGG TTTCCGACCA 1680

GAACTGCAGG CCCCAATCTG TCGTCAGTTG GATCAGATGT TCAATTGCGG GTACTTCTTC 1740

AGGACTGGGT GCACACTGCC ATGCTTTACC ACGTTTATTA TTCAAGACAG GTTCAACCCG 1800

CCCTATTCCC TCAGTGGTGA GCCCGTTGAA GACGGAGTTA CATGCGCGGT TGGGACTAAA 1860

ACAATGGGGG AGGGCATGAG GCAGAAACTA TGGACAATCC TTACGAGCTG CTGGGAGATA 1920

ATTGCTCTTC GGGAAATTAA CGTGACGTTT AACATACTAG GCCAAGGTGA TAATCAGACA 1980

ATCATCATAC ATAAATCTGC AAGCCAAAAT AACCAGCTAT TAGCGGAGCG AGCACTAGGG 2040

GCCCTGTACA AGCATGCTAG ATTAGCTGGC CATAACCTCA AGGTAGAGGA ATGCTGGGTG 2100

TCAGATTGTC TGTATGAGTA TGGAAAGAAG CTTTTCTTCC GTGGTGTACC TGTCCCGGGC 2160

TGTTTGAAGC AGCTCTCACG GGTGACGGAT TCTACTGGAG AGCTATTCCC AAACCTATAC 2220

TCAAAGTTAG CCTGCTTAAC ATCATCGTGT TTAAGCGCAG CGATGGCAGA CACATCTCCA 2280

TGGGTGGCAC TCGCGACAGG TGTCTGTCTG TATCTTATCG AGTTATATGT TGAGCTGCCT 2340

CCAGCAATCA TGCAGGATGA GTCGCTATTG ACGACCCTCT GCCTCGTAGG CCCATCCATT 2400

GGTGGGCTTC CGACCCCTGC AACCCTACCC AGTGTCTTTT TCAGAGGAAT GTCCGACCCA 2460

CTGCCCTTTC AGCTAGCACT CTTGCAGACC CTCATTAAGA CGACAGGGGT GACCTGTAGC 2520

TTGGTGAATC GTGTGGTCAA GTTACGGATA GCACCCTATC CAGACTGGCT CTCTCTAGTG 2580

ACTGACCCGA CCTCACTCAA CATTGCCCAA GTGTACCGGC CAGAACGTCA GATCAGGAGG 2640

TGGATTGAGG AAGCGATAGC GACAAGCTCA CACTCGTCAC GCATAGCAAC TTTCTTCCAG 2700

CAGCCCCTCA CGGAGATGGC TCAGTTGCTT GCGAGGGACC TTTCAACAAT GATGCCTCTT 2760

CGACCCCGGG ATATGTCGGC CTTATTCGCA TTATCAAATG TCGCATACGG TTTAAGCATT 2820

ATAGATCTAT TTCAAAAATC CTCTACCGTT GTTTCTGCAA GTCAAGCTGT CCATATCGAG 2880

GATGTTGCCC TAGAGAGTGT AAGGTATAAG GAATCTATCA TCCAGGGTCT GTTAGACACC 2940

ACTGAGGGGT ATAACATGCA ACCTTATTTG GAAGGTTGCA CTTACCTTGC AGCCAAACAG 3000

TTACGTAGGT TGACATGGGG TCGAGACCTA GTTGGAGTCA CAATGCCGTT TGTTGCCGAG 3060

CAATTCCATC CTCACAGTTC TGTGGGTGCA AAGGCGGAAC TCTACCTCGA CGCTATTATA 3120

TACTGCCCAC AGGAGACATT GCGGTCACAC CATCTGACTA CCAGGGGGGA CCAGCCGCTT 3180

TACCTCGGAT CCAATACGGC TGTCAAGGTC CAGCGAGGTG AGATCACGGG CCTAACAAAG 3240

TCAAGGGCTG CAAATCTAGT CAGGGACACT CTCGTTCTCC ATCAGTGGTA TAAAGTCCGT 3300

AAAGTTACCG ATCCACACTT GAACACCCTC ATGGCACGCT TCTTACTTGA GAAGGGGTAC 3360

ACATCTGACG CTCGACCTAG CATCCAGGGT GGGACCCTCA CGCATCGTCT CCCATCCCGC 3420

GGAGACTCAC GGCAGGGGCT TACTGGGTAT GTAAATATAC TAAGTACGTG GCTTCGATTC 3480

TCAAGTGATT ATCTTCACTC TTTCTCGAAA TCATCAGACG ACTATACAAT CCACTTTCAG 3540

CATGTATTCA CATACGGTTG CCTCTATGCT GATTCGGTGA TTAGATCGGG CGGTGTTATT 3600

TCCACTCCTT ACCTTTTGAG TGCAAGTTGT AAAACATGCT TTGAGAAGAT AGACTCAGAG 3660

GAGTTCGTCC TGGCATGTGA ACCCCAATAC AGGGGTGCTG AGTGGCTGAT ATCAAAGCCA 3720

GTCACTGTCC CTGAGCAGAT AACTGATGCT GAAGTCGAGT TTGACCCCTG TGTGAGTGCG 3780

GGTTATTGTC TCGGGATTCT CATTGGCAAG TCATTCTTAG TTGACATAAG GGCAAGTGGG 3840

CATGATATCA TGGAGCAGCG GACATGGGCT AACCTGGAGA GGTTTTCTGT ATCGGACATG 3900

CAGAAACTTC CGTGGAGTAT TGTAATTCGG TCTCTCTGGA GATTCCTTAT TGGCGCACGG 3960

CTCCTTCAGT TTGAGAAGGC TGGCCTCATT AGAATGCTGT ATGCTGCGAC AGGTCCAACC 4020

CCTAGCTTCC TAATGAAAGT TTTTCAAGAC TCAGCCCTCC TCATGGACTG CGCACCCCTC 4080

GATCGGCTGT CCCCTAGGAT CAACTTTCAT AGTCGGGGAG ACCTCGTTGC TAAGCTTGTT 4140

TTATTGCCCT TCATCAACCC GGGTATAGTG GAGATTGAAG TGTCTGGAAT TAATAGCAAG 4200

TACCATGCAG TATCGGAGGC CAATATGGAT CTGTACATCG CTGCTGCCAA GTCTGTGGGC 4260

GTGAAGCCCA CACAGTTTGT TGAGGAAACA AACGACTTTA CGGCCCGCGG CCACCACCAT 4320

GGTTGTTATT CCCTTTCTTG GTCTAAGTCA CGCAATCAAT CACAGGTCCT AAAGATGGTA 4380

GTACGGAAGC TGAAGCTCTG TGTCCTGTAT ATATACCCCA CAGTCGATCC CGCCGTTGCT 4440

CTCGACCTGT GCCATCTACC AGCATTAACT ATAATCCTAG TGCTCGGCGG TGACCCAGCG 4500

TACTATGAGC GATTACTTGA GATGGACCTG TGCGGGGCTG TGTCAAGTCG AGTCGATATC 4560

CCCCATTCTC TGGCTGGCAG AACGCACAGG GGGTTCGCAG TGGGCCCAGA CGCTGGTCCA 4620

GGTGTAATTA GACTCGACAG GTTAGAGTCA GTTTGTTATG CTCACCCCTG TTTAGAGGAA 4680

CTAGAGTTTA ATGCATATCT AGACTCTGAG TTGGTTGACA TTAGTGATAT GTGCTGCCTC 4740

CCCTTAGCGA CACCCTGTAA GGCCCTTTTC AGGCCAATAT ATCGGAGCTT ACAGTCGTTC 4800

AGGTTAGCCT TAATGGACAA CTATAGTTTT GTCATGGACC TCATTATGAT CCGAGGACTG 4860

GACATTAGGC CTCACCTTGA GGAATTTGAC GAGCTGCTTG TGGTAGGACA GCACATCCTC 4920

GGCCAGCCCG TCCTAGTAGA GGTTGTTTAC TACGTTGGAG TTGTTAGGAA GCGCCCTGTG 4980

TTAGCGAGGC ATCCGTGGTC AGCAGATCTT AAGCGAATTA CTGTGGGGGG GCGGGCTCCC 5040

TGCCCCTCTG CTGCCAGATT GCGTGATGAG GATTGTCAGG GGTCTCTGTT GGTTGGGCTT 5100

CCTGCTGGGT TGACGCAGTT ATTGATAATT GATTA 5135

(11) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1711 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

```
Met Ser Phe His Ala Ser Leu Leu Arg Glu Glu Glu Thr Pro Arg Pro
1               5                  10                  15

Val Ala Gly Ile Asn Arg Thr Asp Gln Ser Leu Lys Asn Pro Leu Leu
            20                  25                  30

Gly Thr Glu Val Ser Phe Cys Leu Lys Ser Ser Ser Leu Pro His His
        35                  40                  45

Val Arg Ala Leu Gly Gln Ile Lys Ala Arg Asn Leu Ala Ser Cys Asp
    50                  55                  60

Tyr Tyr Leu Leu Phe Arg Gln Val Val Leu Pro Pro Glu Val Tyr Pro
65                  70                  75                  80

Ile Gly Val Leu Ile Arg Ala Ala Glu Ala Ile Leu Thr Val Ile Val
                85                  90                  95

Ser Ala Trp Lys Leu Asp His Met Thr Lys Thr Leu Tyr Ser Ser Val
            100                 105                 110

Arg Tyr Ala Leu Thr Asn Pro Arg Val Arg Ala Gln Leu Glu Leu His
        115                 120                 125

Ile Ala Tyr Gln Arg Ile Val Gly Gln Val Ser Tyr Ser Arg Glu Ala
    130                 135                 140

Asp Ile Gly Pro Lys Arg Leu Gly Asn Met Ser Leu Gln Phe Ile Gln
145                 150                 155                 160

Ser Leu Val Ile Ala Thr Ile Asp Thr Thr Ser Cys Leu Met Thr Tyr
                165                 170                 175

Asn His Phe Leu Ala Ala Ala Asp Thr Ala Lys Ser Arg Cys His Leu
            180                 185                 190

Leu Ile Ala Ser Val Val Gln Gly Ala Leu Trp Glu Gln Gly Ser Phe
        195                 200                 205

Leu Asp His Ile Ile Asn Met Ile Asp Ile Ile Asp Ser Ile Asn Leu
    210                 215                 220

Pro His Asp Asp Tyr Phe Thr Ile Ile Lys Ser Ile Phe Pro Tyr Ser
225                 230                 235                 240

Gln Gly Leu Val Met Gly Arg His Asn Val Ser Val Ser Ser Asp Phe
                245                 250                 255

Ala Ser Val Phe Ala Ile Pro Glu Leu Cys Pro Gln Leu Asp Ser Leu
            260                 265                 270

Leu Lys Lys Leu Leu Gln Leu Asp Pro Val Leu Leu Leu Met Val Ser
        275                 280                 285

Ser Val Gln Lys Ser Trp Tyr Phe Pro Glu Ile Arg Met Val Asp Gly
    290                 295                 300

Ser Arg Glu Gln Leu His Lys Met Arg Val Glu Leu Glu Thr Pro Gln
305                 310                 315                 320

Ala Leu Leu Ser Tyr Gly His Thr Leu Leu Ser Ile Phe Arg Ala Glu
                325                 330                 335

Phe Ile Lys Gly Tyr Val Ser Lys Asn Ala Lys Trp Pro Pro Val His
            340                 345                 350

Leu Leu Pro Gly Cys Asp Lys Ser Ile Lys Asn Ala Arg Glu Leu Gly
        355                 360                 365

Arg Trp Ser Pro Ala Phe Asp Arg Arg Trp Gln Leu Phe Glu Lys Val
    370                 375                 380
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Val Ile Leu Arg Ile Ala Asp Leu Asp Met Asp Pro Asp Phe Asn Asp
385 390 395 400

Ile Val Ser Asp Lys Ala Ile Ile Ser Ser Arg Arg Asp Trp Val Phe
405 410 415

Glu Tyr Asn Ala Ala Ala Phe Trp Lys Lys Tyr Gly Glu Arg Leu Glu
420 425 430

Arg Pro Pro Ala Arg Ser Gly Pro Ser Arg Leu Val Asn Ala Leu Ile
435 440 445

Asp Gly Arg Leu Asp Asn Ile Pro Ala Leu Leu Glu Pro Phe Tyr Arg
450 455 460

Gly Ala Val Glu Phe Glu Asp Arg Leu Thr Val Leu Val Pro Lys Glu
465 470 475 480

Lys Glu Leu Lys Val Lys Gly Arg Phe Phe Ser Lys Gln Thr Leu Ala
485 490 495

Ile Arg Ile Tyr Gln Val Val Ala Glu Ala Ala Leu Lys Asn Glu Val
500 505 510

Met Pro Tyr Leu Lys Thr His Ser Met Thr Met Ser Ser Thr Ala Leu
515 520 525

Thr His Leu Leu Asn Arg Leu Ser His Thr Ile Thr Lys Gly Asp Ser
530 535 540

Phe Val Ile Asn Leu Asp Tyr Ser Ser Trp Cys Asn Gly Phe Arg Pro
545 550 555 560

Glu Leu Gln Ala Pro Ile Cys Arg Gln Leu Asp Gln Met Phe Asn Cys
565 570 575

Gly Tyr Phe Phe Arg Thr Gly Cys Thr Leu Pro Cys Phe Thr Thr Phe
580 585 590

Ile Ile Gln Asp Arg Phe Asn Pro Pro Tyr Ser Leu Ser Gly Glu Pro
595 600 605

Val Glu Asp Gly Val Thr Cys Ala Val Gly Thr Lys Thr Met Gly Glu
610 615 620

Gly Met Arg Gln Lys Leu Trp Thr Ile Leu Thr Ser Cys Trp Glu Ile
625 630 635 640

Ile Ala Leu Arg Glu Ile Asn Val Thr Phe Asn Ile Leu Gly Gln Gly
645 650 655

Asp Asn Gln Thr Ile Ile Ile His Lys Ser Ala Ser Gln Asn Asn Gln
660 665 670

Leu Leu Ala Glu Arg Ala Leu Gly Ala Leu Tyr Lys His Ala Arg Leu
675 680 685

Ala Gly His Asn Leu Lys Val Glu Glu Cys Trp Val Ser Asp Cys Leu
690 695 700

Tyr Glu Tyr Gly Lys Lys Leu Phe Phe Arg Gly Val Pro Val Pro Gly
705 710 715 720

Cys Leu Lys Gln Leu Ser Arg Val Thr Asp Ser Thr Gly Glu Leu Phe
725 730 735

Pro Asn Leu Tyr Ser Lys Leu Ala Cys Leu Thr Ser Ser Cys Leu Ser
740 745 750

Ala Ala Met Ala Asp Thr Ser Pro Trp Val Ala Leu Ala Thr Gly Val
755 760 765

Cys Leu Tyr Leu Ile Glu Leu Tyr Val Glu Leu Pro Pro Ala Ile Met
770 775 780

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Gln Asp Glu Ser Leu Leu Thr Thr Leu Cys Leu Val Gly Pro Ser Ile
785                 790                 795                 800

Gly Gly Leu Pro Thr Pro Ala Thr Leu Pro Ser Val Phe Phe Arg Gly
                805                 810                 815

Met Ser Asp Pro Leu Pro Phe Gln Leu Ala Leu Leu Gln Thr Leu Ile
            820                 825                 830

Lys Thr Thr Gly Val Thr Cys Ser Leu Val Asn Arg Val Val Lys Leu
        835                 840                 845

Arg Ile Ala Pro Tyr Pro Asp Trp Leu Ser Leu Val Thr Asp Pro Thr
    850                 855                 860

Ser Leu Asn Ile Ala Gln Val Tyr Arg Pro Glu Arg Gln Ile Arg Arg
865                 870                 875                 880

Trp Ile Glu Glu Ala Ile Ala Thr Ser Ser His Ser Ser Arg Ile Ala
                885                 890                 895

Thr Phe Phe Gln Gln Pro Leu Thr Glu Met Ala Gln Leu Leu Ala Arg
            900                 905                 910

Asp Leu Ser Thr Met Met Pro Leu Arg Pro Arg Asp Met Ser Ala Leu
        915                 920                 925

Phe Ala Leu Ser Asn Val Ala Tyr Gly Leu Ser Ile Ile Asp Leu Phe
    930                 935                 940

Gln Lys Ser Ser Thr Val Val Ser Ala Ser Gln Ala Val His Ile Glu
945                 950                 955                 960

Asp Val Ala Leu Glu Ser Val Arg Tyr Lys Glu Ser Ile Ile Gln Gly
                965                 970                 975

Leu Leu Asp Thr Thr Glu Gly Tyr Asn Met Gln Pro Tyr Leu Glu Gly
            980                 985                 990

Cys Thr Tyr Leu Ala Ala Lys Gln Leu Arg Arg Leu Thr Trp Gly Arg
        995                 1000                1005

Asp Leu Val Gly Val Thr Met Pro Phe Val Ala Glu Gln Phe His Pro
    1010                1015                1020

His Ser Ser Val Gly Ala Lys Ala Glu Leu Tyr Leu Asp Ala Ile Ile
1025                1030                1035                1040

Tyr Cys Pro Gln Glu Thr Leu Arg Ser His His Leu Thr Thr Arg Gly
                1045                1050                1055

Asp Gln Pro Leu Tyr Leu Gly Ser Asn Thr Ala Val Lys Val Gln Arg
            1060                1065                1070

Gly Glu Ile Thr Gly Leu Thr Lys Ser Arg Ala Ala Asn Leu Val Arg
        1075                1080                1085

Asp Thr Leu Val Leu His Gln Trp Tyr Lys Val Arg Lys Val Thr Asp
    1090                1095                1100

Pro His Leu Asn Thr Leu Met Ala Arg Phe Leu Leu Glu Lys Gly Tyr
1105                1110                1115                1120

Thr Ser Asp Ala Arg Pro Ser Ile Gln Gly Gly Thr Leu Thr His Arg
                1125                1130                1135

Leu Pro Ser Arg Gly Asp Ser Arg Gln Gly Leu Thr Gly Tyr Val Asn
            1140                1145                1150

Ile Leu Ser Thr Trp Leu Arg Phe Ser Ser Asp Tyr Leu His Ser Phe
        1155                1160                1165

Ser Lys Ser Ser Asp Asp Tyr Thr Ile His Phe Gln His Val Phe Thr
    1170                1175                1180
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Tyr Gly Cys Leu Tyr Ala Asp Ser Val Ile Arg Ser Gly Gly Val Ile
1185 1190 1195 1200

Ser Thr Pro Tyr Leu Leu Ser Ala Ser Cys Lys Thr Cys Phe Glu Lys
1205 1210 1215

Ile Asp Ser Glu Glu Phe Val Leu Ala Cys Glu Pro Gln Tyr Arg Gly
1220 1225 1230

Ala Glu Trp Leu Ile Ser Lys Pro Val Thr Val Pro Glu Gln Ile Thr
1235 1240 1245

Asp Ala Glu Val Glu Phe Asp Pro Cys Val Ser Ala Gly Tyr Cys Leu
1250 1255 1260

Gly Ile Leu Ile Gly Lys Ser Phe Leu Val Asp Ile Arg Ala Ser Gly
1265 1270 1275 1280

His Asp Ile Met Glu Gln Arg Thr Trp Ala Asn Leu Glu Arg Phe Ser
1285 1290 1295

Val Ser Asp Met Gln Lys Leu Pro Trp Ser Ile Val Ile Arg Ser Leu
1300 1305 1310

Trp Arg Phe Leu Ile Gly Ala Arg Leu Leu Gln Phe Glu Lys Ala Gly
1315 1320 1325

Leu Ile Arg Met Leu Tyr Ala Ala Thr Gly Pro Thr Pro Ser Phe Leu
1330 1335 1340

Met Lys Val Phe Gln Asp Ser Ala Leu Leu Met Asp Cys Ala Pro Leu
1345 1350 1355 1360

Asp Arg Leu Ser Pro Arg Ile Asn Phe His Ser Arg Gly Asp Leu Val
1365 1370 1375

Ala Lys Leu Val Leu Leu Pro Phe Ile Asn Pro Gly Ile Val Glu Ile
1380 1385 1390

Glu Val Ser Gly Ile Asn Ser Lys Tyr His Ala Val Ser Glu Ala Asn
1395 1400 1405

Met Asp Leu Tyr Ile Ala Ala Ala Lys Ser Val Gly Val Lys Pro Thr
1410 1415 1420

Gln Phe Val Glu Glu Thr Asn Asp Phe Thr Ala Arg Gly His His His
1425 1430 1435 1440

Gly Cys Tyr Ser Leu Ser Trp Ser Lys Ser Arg Asn Gln Ser Gln Val
1445 1450 1455

Leu Lys Met Val Val Arg Lys Leu Lys Leu Cys Val Leu Tyr Ile Tyr
1460 1465 1470

Pro Thr Val Asp Pro Ala Val Ala Leu Asp Leu Cys His Leu Pro Ala
1475 1480 1485

Leu Thr Ile Ile Leu Val Leu Gly Gly Asp Pro Ala Tyr Tyr Glu Arg
1490 1495 1500

Leu Leu Glu Met Asp Leu Cys Gly Ala Val Ser Ser Arg Val Asp Ile
1505 1510 1515 1520

Pro His Ser Leu Ala Gly Arg Thr His Arg Gly Phe Ala Val Gly Pro
1525 1530 1535

Asp Ala Gly Pro Gly Val Ile Arg Leu Asp Arg Leu Glu Ser Val Cys
1540 1545 1550

Tyr Ala His Pro Cys Leu Glu Glu Leu Glu Phe Asn Ala Tyr Leu Asp
1555 1560 1565

Ser Glu Leu Val Asp Ile Ser Asp Met Cys Cys Leu Pro Leu Ala Thr
1570 1575 1580

Pro Cys Lys Ala Leu Phe Arg Pro Ile Tyr Arg Ser Leu Gln Ser Phe
1585 1590 1595 1600

Arg Leu Ala Leu Met Asp Asn Tyr Ser Phe Val Met Asp Leu Ile Met
1605 1610 1615

Ile Arg Gly Leu Asp Ile Arg Pro His Leu Glu Glu Phe Asp Glu Leu
1620 1625 1630

Leu Val Val Gly Gln His Ile Leu Gly Gln Pro Val Leu Val Glu Val
1635 1640 1645

Val Tyr Tyr Val Gly Val Val Arg Lys Arg Pro Val Leu Ala Arg His
1650 1655 1660

Pro Trp Ser Ala Asp Leu Lys Arg Ile Thr Val Gly Gly Arg Ala Pro
1665 1670 1675 1680

Cys Pro Ser Ala Ala Arg Leu Arg Asp Glu Asp Cys Gln Gly Ser Leu
1685 1690 1695

Leu Val Gly Leu Pro Ala Gly Leu Thr Gln Leu Leu Ile Ile Asp
1700 1705 1710

(12) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GCCTCCCCTT AGCGACACCC TGTA 24

(13) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

GAAACATATC GCGCCGTGCA 20

(14) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

TACGTTGGAG TTGTTAGGAA GC 22

(15) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

GAGCTTAGGG AGGCTCGCTG 20

(16) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

TCCTCGAGAT GAATTCAAAA CATTCCTATC 30

(17) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

CTAAGGCCCT GAAGATCGAA T 21

(18) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

CCCTCGAGGA CCAAGATTT 19

(19) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

AGAATCATAT GGCAACGCGA CCATC 25

(20) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8910 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

GTTGCGTTAA CAACAAACCA CTCATCATTC TTCTAACAAA ATGAACACAC GCAATGCCAC 60

CCAAGAGACG CCTGGTTGAT GACGCCGATG CCATGGAGGA TCAAGATCTA TATGAACCCC 120

CAGCGAGCCT CCCTAAGCTC CCTGGGAAAT TCCTACAATA CACCGTTGGG GGGTCTGACC 180

CGCATCCGGG TATAGGGCAT GAGAAAGACA TCAGGCAGAA CGCAGTGGCA TTGTTAGACC 240

AGTCACGGCG CGATATGTTT CACACAGTAA CGCCTAGCCT TGTGTTTCTA TGTTTGCTAA 300

TCCCAGGACT GCACGCTGCG TTTGTTCACG GAGGGGTGCC TCGTGAATCC TACCTGTCGA 360

CGCCTGTCAC GCGTGGAGAA CAGACTGTTG TTAAGACTGC GAAGTTTTAC GGGGAAAAGA 420

CGACGCAGCG TGATCTCACC GAGCTGGAGA TCTCCTCTAT CTTCAGCCAT TGTTGCTCAT 480

TACTAATAGG GGTTGTGATA GGATCGTCGT CTAAGATCAA AGCAGGAGCC GAGCAGATCA 540

AGAAAAGGTT TAAAACTATG ATGGCAGCCT TAAACCGGCC ATCCCATGGT GAGACTGCTA 600

CACTACTCCA GATGTTTAAT CCACATGAGG CTATAGATTG GATTAACGGC CAACCCTGGG 660

TAGGCTCCTT TGTGTTGTCT CTACTAACTA CAGACTTTGA GTCCCCAGGT AAAGAATTTA 720

TGGACCAGAT TAAGCTTGTC GCAAGTTATG CACAGATGAC TACGTACACT ACTATAAAGG 780

AGTACCTCGC AGAATGCATG GATGCTACCC TTACAATCCC CGTAGTTGCA TATGAGATCC 840

GTGACTTTTT AGAAGTTTCA GCAAAGCTTA AGGAGGATCA TGCTGACCTG TTCCCGTTTC 900

TGGGGGCCAT TAGACACCCC GACGCTATCA AGCTGGCGCC ACGAAGCTTT CCCAATCTGG 960

CCTCCGCAGC GTTTTACTGG AGTAAGAAGG AAAACCCCAC AATGGCAGGC TACCGGGCCT 1020

CCACCATCCA GCCGGGCGCA AGTGTCAAGG AAACCCAGCT TGCCCGGTAT AGGCGCCGCG 1080

AGATATCTCG TGGAGAGGAC GGGGCAGAGC TCTCAGGTGA GATCTCTGCC ATAATGAAGA 1140

TGATAGGTGT GACTGGTCTA AACTAAAAAA CAATGAACAA ACCAATAAAA AACCAAATGC 1200

GGCAAACCCT CCGCGACCTG CGATGAGCTC CGACCTCCGG CTGACATTGC TTGAACTAGT 1260

CAGGAGGCTC AATGGCAACG CGACCATCGA GTCTGGTCGA CTCCCTGGAG GACGAAGAAG 1320

ATCCCCAGAC ACTACGACGG GAACGACCGG GGTCACCAAG ACCACGGAAG GTCCCAAGGA 1380

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TCTCAGACCC AGACCAGCGA ACCGGAAGGG AGCAGCTGTC GAATGATGAG CTAATCAAGA 1500

AGTTAGTGAC GGAGCTGGCC GAGAATAGCA TGATCGAGGC TGAGGAGGTG CGGGGCACTC 1560

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ACATCAAGAT ACTAGATCGC TCCATGAAGA CAATGATGGA GACAATGAAG CTCATGATGG 1740

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CCCATCCTGC ACCTCCGCGC ATTTATCCCC AGCTCCCAAG TGCCCCGACA ACGGATGAAT 1860

GGGACATCAT ACCATAAAAA AATCGAATCA CCATGAATTC AAAACATTCC TATGTGGAGC 1920

TCAAGGACAA GGTAATCGTC CCTGGATGGC CCACACTGAT GCTTGAGATA GACTTTGTAG 1980

GGGGGACTTC ACGGAACCAG TTCCTTAACA TCCCATTTCT TTCAGTGAAA GAGCCTCTGC 2040

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GCAAGCACGC AATCAATGCA GCCTTCAATG TCTTTTCTTA TCGGCTTCGG AACATTGGTG 2280

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TCATGCGAGG TTAGTTATCT CAACCACACG ACTATTAGCC TCCCGGCAGT CCACACATCA 2460

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CCCTTCGAGT GCAACTGGTT CTACTGCTGC TCGGCGATTA CAACAGAGAT CTGCCGATGC 2640

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CGGTGATTAG ATCGGGCGGT GTTATTTCCA CTCCTTACCT TTTGAGTGCA AGTTGTAAAA 7320

CATGCTTTGA GAAGATAGAC TCAGAGGAGT TCGTCCTGGC ATGTGAACCC CAATACAGGG 7380

GTGCTGAGTG GCTGATATCA AAGCCAGTCA CTGTCCCTGA GCAGATAACT GATGCTGAAG 7440

TCGAGTTTGA CCCCTGTGTG AGTGCGGGTT ATTGTCTCGG GATTCTCATT GGCAAGTCAT 7500

TCTTAGTTGA CATAAGGGCA AGTGGGCATG ATATCATGGA GCAGCGGACA TGGGCTAACC 7560

TGGAGAGGTT TTCTGTATCG GACATGCAGA AACTTCCGTG GAGTATTGTA ATTCGGTCTC 7620

TCTGGAGATT CCTTATTGGC GCACGGCTCC TTCAGTTTGA GAAGGCTGGC CTCATTAGAA 7680

TGCTGTATGC TGCGACAGGT CCAACCCCTA GCTTCCTAAT GAAAGTTTTT CAAGACTCAG 7740

CCCTCCTCAT GGACTGCGCA CCCCTCGATC GGCTGTCCCC TAGGATCAAC TTTCATAGTC 7800

GGGGAGACCT CGTTGCTAAG CTTGTTTTAT TGCCCTTCAT CAACCCGGGT ATAGTGGAGA 7860

TTGAAGTGTC TGGAATTAAT AGCAAGTACC ATGCAGTATC GGAGGCCAAT ATGGATCTGT 7920

ACATCGCTGC TGCCAAGTCT GTGGGCGTGA AGCCCACACA GTTTGTTGAG GAAACAAACG 7980

ACTTTACGGC CCGCGGCCAC CACCATGGTT GTTATTCCCT TTCTTGGTCT AAGTCACGCA 8040

ATCAATCACA GGTCCTAAAG ATGGTAGTAC GGAAGCTGAA GCTCTGTGTC CTGTATATAT 8100

ACCCCACAGT CGATCCCGCC GTTGCTCTCG ACCTGTGCCA TCTACCAGCA TTAACTATAA 8160

TCCTAGTGCT CGGCGGTGAC CCAGCGTACT ATGAGCGATT ACTTGAGATG GACCTGTGCG 8220

GGGCTGTGTC AAGTCGAGTC GATATCCCCC ATTCTCTGGC TGGCAGAACG CACAGGGGGT 8280

TCGCAGTGGG CCCAGACGCT GGTCCAGGTG TAATTAGACT CGACAGGTTA GAGTCAGTTT 8340

GTTATGCTCA CCCCTGTTTA GAGGAACTAG AGTTTAATGC ATATCTAGAC TCTGAGTTGG 8400

TTGACATTAG TGATATGTGC TGCCTCCCCT TAGCGACACC CTGTAAGGCC CTTTTCAGGC 8460

CAATATATCG GAGCTTACAG TCGTTCAGGT TAGCCTTAAT GGACAACTAT AGTTTTGTCA 8520

TGGACCTCAT TATGATCCGA GGACTGGACA TTAGGCCTCA CCTTGAGGAA TTTGACGAGC 8580

TGCTTGTGGT AGGACAGCAC ATCCTCGGCC AGCCCGTCCT AGTAGAGGTT GTTTACTACG 8640

TTGGAGTTGT TAGGAAGCGC CCTGTGTTAG CGAGGCATCC GTGGTCAGCA GATCTTAAGC 8700

GAATTACTGT GGGGGGGCGG GCTCCCTGCC CCTCTGCTGC CAGATTGCGT GATGAGGATT 8760

GTCAGGGGTC TCTGTTGGTT GGGCTTCCTG CTGGGTTGAC GCAGTTATTG ATAATTGATT 8820

AAGATCAAGC CACCTACTAC CCTATTCTTA AAAAACCATA TGTCAGTGGT GCAGTGCTTG 8880

GGCTTGGTTG TTGCTTTGTT GTAGCGCGTT 8910

(21) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Met Ala Thr Arg Pro Ser Ser Leu
1 5

(22) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Asn Ala Leu Thr Gln Pro Val Asp Gln Leu Leu Lys
1 5 10

(23) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Asp Gln Pro Thr Gly Arg Glu Gln
1 5

(24) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Val Arg Gly Thr Leu Gly Asp Ile
1 5

(25) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

Thr Ala Gln Arg Cys Asp His Ser
1 5

(26) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

Met Glu Thr Met Lys Leu Met Met Glu Lys Val Asp
1 5 10

(27) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

Pro Met Leu Pro Ser His Pro Ala
1 5

(28) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

Thr Ala Asp Glu Trp Asp Ile Ile
1 5

(29) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

Met Asn Ser Lys His Ser Tyr Val
1 5

(30) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

Thr Leu Met Leu Glu Ile Asp Phe
1 5

(31) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

Gly His Ser Leu Val Asn Ile Tyr Phe Gln Ile Asp
1 5 10

(32) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

Tyr Lys Asp Pro Ile Arg Lys Tyr
1 5

(33) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

Ala Phe Asn Val Phe Ser Tyr Arg
1 5

(34) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2658 amino acids
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

GGTAGACCAG CTCCTGAAGG ACCTCAGGAA GAACCCCTCC ATGATCTCAG 50

ACCCAGACCA GCGAACCGGA AGGGAGCAGC TATCGAATGA TGAGCTTATC 100

AAGAAGCTAG TGACGGAGCT GGCCGAGAAT AGCATGATCG AGGCTGAGGA 150

GGTGCGGGGC ACTCTTGGGG ACATCTCGGC TCGCATCGAG GCAGGGTTTG 200

AGTCCCTGTC CGCCCTCCAA GTGGAAACCA TCCAGACAGC TCAGCGGTGC 250

GACCACTCCG ATAGCATCAG AATCCTTGGC GAGAACATCA AGATACTGGA 300

TCGCTCCATG AAGACAATGA TGGAGACAAT GAAGCTCATG ATGGAGAAGG 350

TGGACCTCCT CTACGCATCA ACCGCCGTTG GGACCTCTGC ACCCATGTTG 400

CCCTCCCATC CTGCACCTCC GCGCATTTAT CCCCAGCTCC CAAGTGCCCC 450

GACAGCGGAT GAGTGGGACA TCATACCATA AAAAAATCGA ATCACCATGA 500

ATTCAAAGCA TTCCTATGTG GAGCTCAAGG ACAAGGTAAT CGTCCCTGGA 550

TGGCCCACAC TGATGCTTGA GATAGACTTT GTAGGAGGGA CTTCACGGAA 600

CCAGTTCCTT AACATCCCAT TTCTTTCAGT GAAAGAGCCT CTGCAGCTTC 650

CACGCGAGAA GAAGTTGACC GACTACTTCA CCATTGACGT AGAGCCAGCA 700

GGTCATTCCC TGGTCAACAT ATACTTCCAG ATTGACGACT TCTTGCTCCT 750

AACACTCAAC TCACTGTCCG TATACAAGGA CCCGATTAGG AAATACATGT 800

TCCTACGCCT CAACAAGGAA CAGAGCAAGC ACGCAATTAA TGCAGCTTTC 850

AATGTCTTCT CTTATCGGCT TCGGAACATT GGTGTTGGCC CTCTCGGCCC 900

AGACATTCGA TCTTCAGGGC CTTAGTTGCA ATACTGACTC CACTCCTGGA 950

TTAATCGATC TGGAGATAAG GCGACTTTGC CACACCCCAA CGGAAAATGT 1000

CATTTCATGC GAGGTTAGTT ATCTTAACCA CACGACTATT AGCCTCCCGG 1050

CAGTCCACAC GTCATGCCTC AAGTACCACT GCAAAACCTA TTGGGGATTC 1100

TTTGGTAGCT ACAGCGCTGA CCGAATCATC AATCGGTACA CTGGTACTGT 1150

TAAGGGTTGT TTAAACAACT CAGCGCCAGA GGATCCCTTC GAGTGCAACT 1200

GGTTCTACTG CTGCTCGGCG ATTACAACAG AGATCTGCCG ATGCTCTATT 1250

ACAAATGTCA CGGTGGCTGT ACAGACATTC CCACCGTTCA TGTACTGCAG 1300

TTTCGCGGAC TGTAGTACTG TGAGTCAGCA GGAGCTAGAG AGTGGAAAGG 1350

CAATGCTGAG CGATGGCAGT ACCTTAACTT ATACCCCGTA TATCTTACAA 1400

TCAGAAGTCG TGAACAAAAC CCTTAATGGG ACTATACTCT GCAACTCATC 1450

CTCCAAGATA GTTTCCTTCG ATGAATTTAG GCGTTCATAC TCCCTAGCGA 1500

ATGGTAGTTA CCAGAGCTCA TCAATCAATG TGACGTGTGT AAACTACACG 1550

TCGTCCTGCC GGTCCAAGTT GAGAAGGCGG CGTAGGGATA CTCAACAGAT 1600

TGAGTACCTA GTTCACAAGC TTAGGCCTAC ACTGAAAGAT GCGTGGGAGG 1650

ACTGTGAGAT CCTCCAGTCT CTGCTCCTAG GGGTGTTTGG TACTGGGATT 1700

GCAAGTGCTT CGCAATTCTT GAGGGGCTGG CTCAACCACC CTGATATCAT 1750

CGGGTATATA GTTAATGGAG TTGGGGTAGT CTGGCAATGC CATCGTGTTG 1800

ATGTCACGTT CATGGCGTGG AATGAGTCCA CATATTACCC TCCAGTAGAT 1850

TACAATGGAC GGAAGTACTT TCTGAATGAT GAGGGGAGGC TACAAACAAA 1900

CACCCCCGAG GCAAGGCCAG GGCTTAAGCG GGTCATGTGG TTCGGCAGGT 1950

ACTTCCTAGG GACAGTAGGG TCTGGGGTGA AACCGAGGAG GATTCGGTAC 2000

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CATGACCCCC CAGACCAGTA TCGCCTCGGG TCATGAGACA GACCCCATAA 2100

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TTACCAGCCT CCTTCGCGAG GAGGAGACGC CTCGGCCGGT GGCAGGAATA 2350

AACCGTACCG ACCAATCTCT TAAAAACCCT CTTCTCGGGA CAGAGGTCTC 2400

TTTCTGCCTT AAATCGAGTT CACTCCCCCA TCACGTACGA GCATTGGGCC 2450

AGATTAAAGC AAAGAACCTG GCATCCTGTG ACTATTACTT GCTATTCCGC 2500

CAAGTTGTAT TGCCCCCTGA AGTATATCCC ATTGGTGTCT TAATAAGAGC 2550

TGCGGAGGCC ATACTAACAG TTATAGTATC AGCTTGGAAG CTGGATCACA 2600

TGACAAAGAC CCTATACTCC TCTGTGAGAT ATGCACTCAC CAATCCCCGG 2650

GTCCGGGC 8

(35) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

CGCAATCAAT GCAGC 15

(36) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(B) TYPE: amino acid

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

TTCCTGCCAC CGGCCG 16

We claim:

1. A nucleotide sequence selected from the group consisting of:

a) SEQ ID NOs. 5, 7, 9, 19, 33; and their complementary nucleotide sequences;

b) nucleotide sequences capable of hybridizing to SEQ ID NOs. 5, 7, 9, 19, 33 and their complementary nucleotide sequences, wherein said nucleotide sequence is at least 10 nucleotides in length and is not SEQ ID NOs. 1 or 3; and

c) nucleotide sequence fragments selected from the group consisting of: fragments of SEQ ID NOs. 5, 7, and 9; nucleotide number 1 through 53, and nucleotide number 1880 through 8910 of SEQ ID NO 19; complementary nucleotide sequences of the foregoing; and nucleotide sequences capable of hybridizing to the foregoing; wherein each of said fragment encodes a protein comprising at least four amino acids or at least one immunogenic epitope.

2. An amino acid sequence selected from the group consisting of: SEQ ID NOs 6, 8, 10, 20 to 32; amino acid position 1 to amino acid position 102 of p23; amino acid position 37 to amino acid position 201 of p23; amino acid position 102 to amino acid position 201 of p23; amino acid position 154 to amino acid position 201 of p23; amino acid position 1 to amino acid position 70 of unglycosylated gp18; amino acid position 23 to amino acid position 142 of unglycosylated gp18; amino acid position 63 to amino acid position 142 of unglycosylated gp18; amino acid position 100 to amino acid position 142 of unglycosylated gp18; SEQ ID Nos. 20 to 32; a fragment of a full length amino acid sequence of p23, said fragment is selected from the group consisting of: amino acid position 1 to amino acid position 102 of p23, amino acid position 37 to amino acid position 201 of p23, amino acid

position 102 to amino acid position 201 of p23, amino acid position 154 to amino acid position 201 of p23; a fragment of a full length amino acid sequence of gp18, said fragment is selected from the group consisting of: amino acid position 1 to amino acid position 70 of gp18, amino acid position 23 to amino acid position 142 of gp18, amino acid position 63 to amino acid position 142 of gp18, amino acid position 100 to amino acid position 142 of gp18; fragments of the foregoing comprising at least four amino acids or at least one immunogenic epitope; variants and equivalent amino acid sequences of the foregoing.

3. A substantially pure composition of protein gp18, p57, or pol.

4. A composition comprising: recp18, recp57, recpol, a recombinantly produced Borna disease virus, or a recombinantly produced virus encoded by the nucleotide sequence group consisting of: SEQ ID NOs. 5, 7, 9, 19, 33; and their complementary nucleotide sequences.

5. A recombinant cell, virus, or vector, containing a nucleotide sequence of claim 1, or a nucleotide sequence encoding the amino acid sequence of claims 2, 3, or 4.

6. An infective but replication deficient viral vector produced by removing or mutating at least one gene responsible for replication from a nucleotide sequence selected from the group consisting of SEQ ID No. 19, and a nucleotide sequence complementary or hybridizable to SEQ ID No. 19.

7. A method of delivering a desired gene or desired chemical into a host, comprising the step of administering into the host the viral vector of claim 6, or transplanting into the host a cell containing the viral vector of claim 6, said viral vector further comprising the desired gene, or a gene encoding the desired chemical.

8. A method for detecting, in a sample, a ligand capable of binding one or more proteins selected from the group consisting of: gp18, recp18, p57, recp57, pol, recpol, proteins having the amino acid sequences of claim 2, and immunogenic fragments thereof, said method comprising the steps of:

a) contacting the sample with one or more of the proteins; and

b) determining binding of the ligand to the proteins.

9. The method of claim 8, wherein the method is an immunoassay method, the ligand is an antibody immunoreactive with Borna disease virus, the proteins are prebound to a solid support without gel size fractionation.

10. A method for selecting for a ligand capable of binding one or more protein(s) selected from the group consisting of: gp18; recp18; p57; recp57; pol; recpol; proteins having the amino acid sequences of claim 2; and immunogenic fragments of the foregoing; said method comprising the steps of:

a) contacting the ligand with the protein(s); and

b) selecting for the ligand which binds to the protein(s).

11. The method of claim 10, wherein the ligand is a therapeutic agent against a disease selected from the group consisting of: (1) BDV infection or related pathogenesis; and (2) neurologic and neuropsychiatric disease not due to BDV infection, said method further comprising the steps of:

a) treating patients suffering from the disease, with the selected ligand and assaying for efficacy of the selected ligand for combating the disease; and

b) selecting the ligand which is efficacious against the disease, said ligand serving as the therapeutic agent.

12. A ligand capable of binding a protein selected from the group consisting: gp18, p57, recp18, recp57, pol, recpol, proteins having the amino acid sequences of claim 2, and fragments thereof which contain at least one immunogenic epitope.

13. An assay panel comprising one or more proteins selected from the group selected from the group consisting of: gp18; recp18; p57; recp57; pol; recpol; proteins having the amino acid sequences of claim 2; and immunogenic fragments of the foregoing.

14. A method for detecting a nucleotide sequence in a sample comprising the steps of:

(a) contacting the sample to a test nucleotide sequence selected from the group consisting of: nucleotide sequences encoding genome of Borna disease virus, gp18, p57, and pol; fragments of genome of Borna disease virus which are at least 10 nucleotides in length and between nucleotide number 1 through 53, and nucleotide number 1880 through 8910 of SEQ ID NO 19; fragments of nucleotide sequences encoding gp18, p57, pol, which are at least 10 nucleotides in length; and

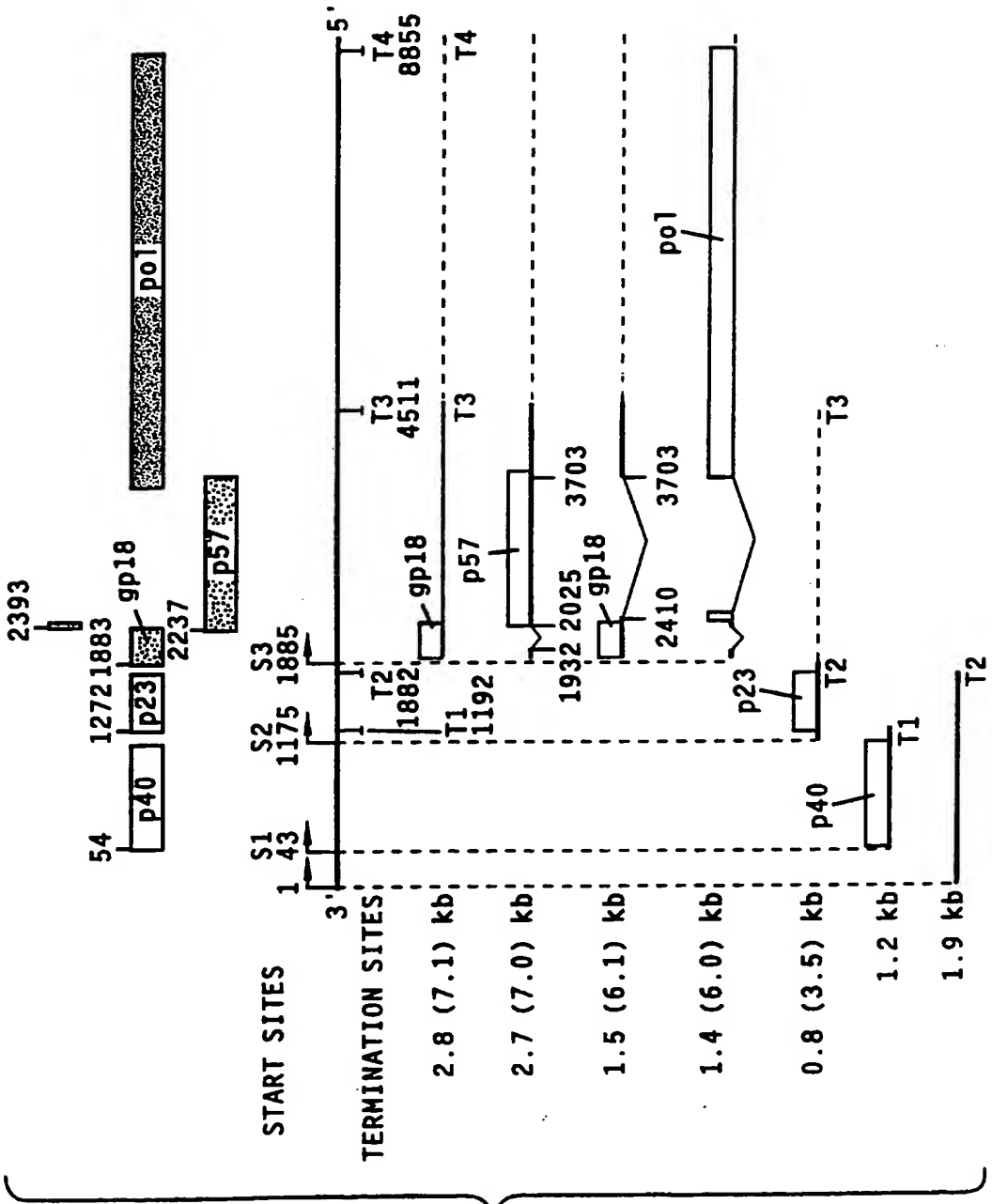
nucleotide sequences which are complementary or hybridizable to any of the foregoing nucleotide sequences;

(b) incubating the sample with the test nucleotide sequence under hybridization condition; and

(c) detecting hybridization of any nucleotide sequence in the sample with the test nucleotide sequence.

15. A method for treating a patient suffering from: (1) BDV infection or related pathogenesis; or (2) neurologic and neuropsychiatric disease not due to BDV infection, said method comprising the steps of administering to the patient a therapeutic amount of the therapeutic agent of claim 11.

16. A vaccine comprising gp18, recp18, p57, recp57, pol, recpol, p23, recp23, p40, recp40, recombinantly produced Borna disease virus, the proteins of claim 2, fragments of the foregoing, or their encoding nucleotide sequences, capable of provoking cellular or humoral immune response in an organism administered with the vaccine.


2393
1272 1883
gp18
2237
p57
pol
54
p40
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S1
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1.2 kb
1.9 kb

FIG. 1

FIG. 2(1)

```
1    GTTGCGTTAACAACAAACCACTCATCATTCTTCTAACAAAATGAACACACGCAATGCCACCCAAGAGACGCCTGGTTGATGACGCCGATGCCATGGAGGA  100
                                                                M  P  P  K  R  R  L  V  D  D  A  D  A  M  E  D

101  TCAAGATCTATATGAACCCCCAGCGAGCCTCCCTAAGCTCCCTGGGAAATTCCTACAATACACCGTTGGGGGGTCTGACCCGCATCCGGGTATAGGGCAT  200
       Q  D  L  Y  E  P  P  A  S  L  P  K  L  P  G  K  F  L  Q  Y  T  V  G  G  S  D  P  H  P  G  I  G  H

201  GAGAAAGACATCAGGCAGAACGCAGTGGCATTGTTAGACCAGTCACGGCGCGATATGTTTCACACAGTAACGCCTAGCCTTGTGTTTCTATGTTTGCTAA  300
       E  K  D  I  R  Q  N  A  V  A  L  L  D  Q  S  R  R  D  M  F  H  T  V  T  P  S  L  V  F  L  C  L  L  I

301  TCCCAGGACTGCACGCTGCGTTTGTTCACGGAGGGGTGCCTCGTGAATCCTACCTGTCGACGCCTGTCACGCGTGGAGAACAGACTGTTGTTAAGACTGC  400
       P  G  L  H  A  A  F  V  H  G  G  V  P  R  E  S  Y  L  S  T  P  V  T  R  G  E  Q  T  V  V  K  T  A

401  GAAGTTTTACGGGGAAAAGACGACGCAGCGTGATCTCACCGAGCTGGAGATCTCCTCTATCTTCAGCCATTGTTGCTCATTACTAATAGGGGTTGTGATA  500
       K  F  Y  G  E  K  T  T  Q  R  D  L  T  E  L  E  I  S  S  I  F  S  H  C  C  S  L  L  I  G  V  V  I

501  GGATCGTCGTCTAAGATCAAAGCAGGAGCCGAGCAGATCAAGAAAAGGTTTAAAACTATGATGGCAGCCTTAAACCGGCCATCCCATGGTGAGACTGCTA  600
       G  S  S  S  K  I  K  A  G  A  E  Q  I  K  K  R  F  K  T  M  M  A  A  L  N  R  P  S  H  G  E  T  A  T

601  CACTACTCCAGATGTTTAATCCACATGAGGCTATAGATTGGATTAACGGCCAACCCTGGGTAGGCTCCTTTGTGTTGTCTCTACTAACTACAGACTTTGA  700
       L  L  Q  M  F  N  P  H  E  A  I  D  W  I  N  G  Q  P  W  V  G  S  F  V  L  S  L  L  T  T  D  F  E

701  GTCCCCAGGTAAAGAATTTATGGACCAGATTAAGCTTGTCGCAAGTTATGCACAGATGACTACGTACACTACTATAAAGGAGTACCTCGCAGAATGCATG  800
       S  P  G  K  E  F  M  D  Q  I  K  L  V  A  S  Y  A  Q  M  T  T  Y  T  T  I  K  E  Y  L  A  E  C  M

801  GATGCTACCCTTACAATCCCCGTAGTTGCATATGAGATCCGTGACTTTTTAGAAGTTTCAGCAAAGCTTAAGGAGGATCATGCTGACCTGTTCCCGTTTC  900
       D  A  T  L  T  I  P  V  V  A  Y  E  I  R  D  F  L  E  V  S  A  K  L  K  E  D  H  A  D  L  F  P  F  L

901  TGGGGGCCATTAGACACCCCGACGCTATCAAGCTGGCGCCACGAAGCTTTCCCAATCTGGCCTCCGCAGCGTTTTACTGGAGTAAGAAGGAAAACCCCAC  1000
       G  A  I  R  H  P  D  A  I  K  L  A  P  R  S  F  P  M  L  A  S  A  A  F  Y  W  S  K  K  E  N  P  T

1001 AATGGCAGGCTACCGGGCCTCCACCATCCAGCCGGGCGCAAGTGTCAAGGAAACCCAGCTTGCCCGGTATAGGCGCCGCGAGATATCTCGTGGAGAGGAC  1100
       M  A  G  Y  R  A  S  T  I  Q  P  G  A  S  V  K  E  T  Q  L  A  R  Y  R  R  R  E  I  S  R  G  E  D

1101 GGGGCAGAGCTCTCAGGTGAGATCTCTGCCATAATGAAGATGATAGGTGTGACTGGTCTAAACTAAAAAACAATGAACAAACCAATAAAAAACCAAATGC  1200
       G  A  E  L  S  G  E  I  S  A  I  M  K  M  I  G  V  T  G  L  N  *

1201 GGCAAACCCTCCGCGACCTGCGATGAGCTCCGACCTCCGGCTGACATTGCTTGAACTAGTCAGGAGGCTCAATGGCAACGCGACCATCGAGTCTGGTCGA  1300
                                                                M  A  T  R  P  S  S  L  V  D

1301 CTCCCTGGAGGACGAAGAAGATCCCCAGACACTACGACGGGAACGACCGGGGTCACCAAGACCACGGAAGGTCCCAAGGAATGCATTGACCCAACCAGTA  1400
       S  L  E  D  E  E  D  P  Q  T  L  R  R  E  R  P  G  S  P  R  P  R  K  V  P  R  M  A  L  T  Q  P  V

1401 GACCAGCTCCTGAAGGACCTCAGGAAGAACCCCTCCATGATCTCAGACCCAGACCAGCGAACCGGAAGGGAGCAGCTGTCGAATGATGAGCTAATCAAGA  1500
       D  Q  L  L  K  D  L  R  K  N  P  S  M  I  S  D  P  D  Q  R  T  G  R  E  Q  L  S  N  D  E  L  I  K  K

1501 AGTTAGTGACGGAGCTGGCCGAGAATAGCATGATCGAGGCTGAGGAGGTGCGGGGCACTCTTGGAGACATCTCGGCTCGTATCGAGGCAGGGTTTGAGTC  1600
       L  V  T  E  L  A  E  N  S  M  I  E  A  E  E  V  R  G  T  L  G  D  I  S  A  R  I  E  A  G  F  E  S

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       L  S  A  L  Q  V  E  T  I  Q  T  A  Q  R  C  D  H  S  D  S  I  R  I  L  G  E  N  I  K  I  L  D  R

1701 TCCATGAAGACAATGATGGAGACAATGAAGCTCATGATGGAGAAGGTGGATCTCCTCTACGCATCAACCGCCGTTGGGACCTCTGCACCCATGTTGCCCT  1800
       S  M  K  T  M  M  E  T  M  K  L  M  M  E  K  V  D  L  L  Y  A  S  T  A  V  G  T  S  A  P  M  L  P  S
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FIG. 2(2)

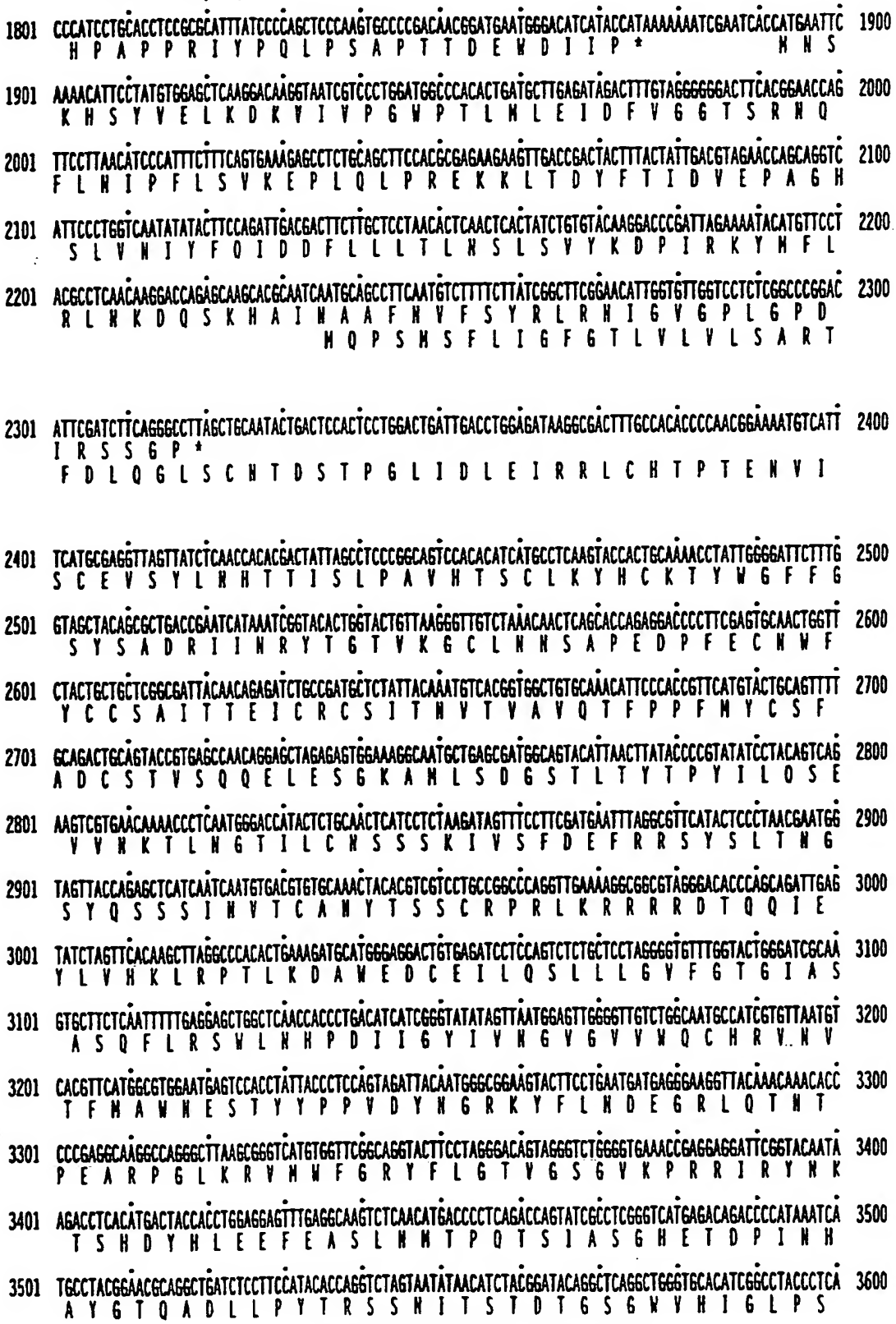
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FIG. 2(3)

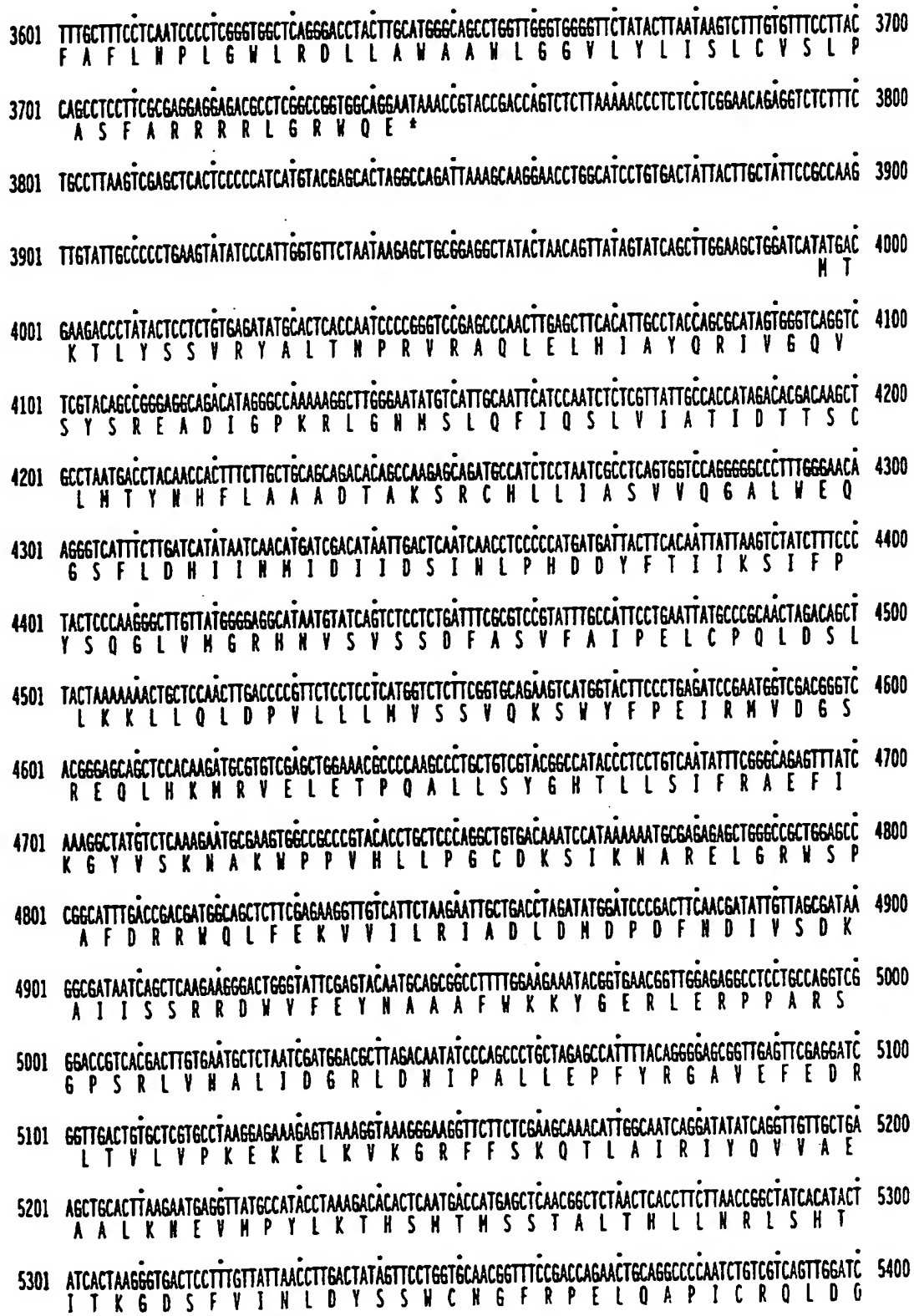
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FIG. 2(4)

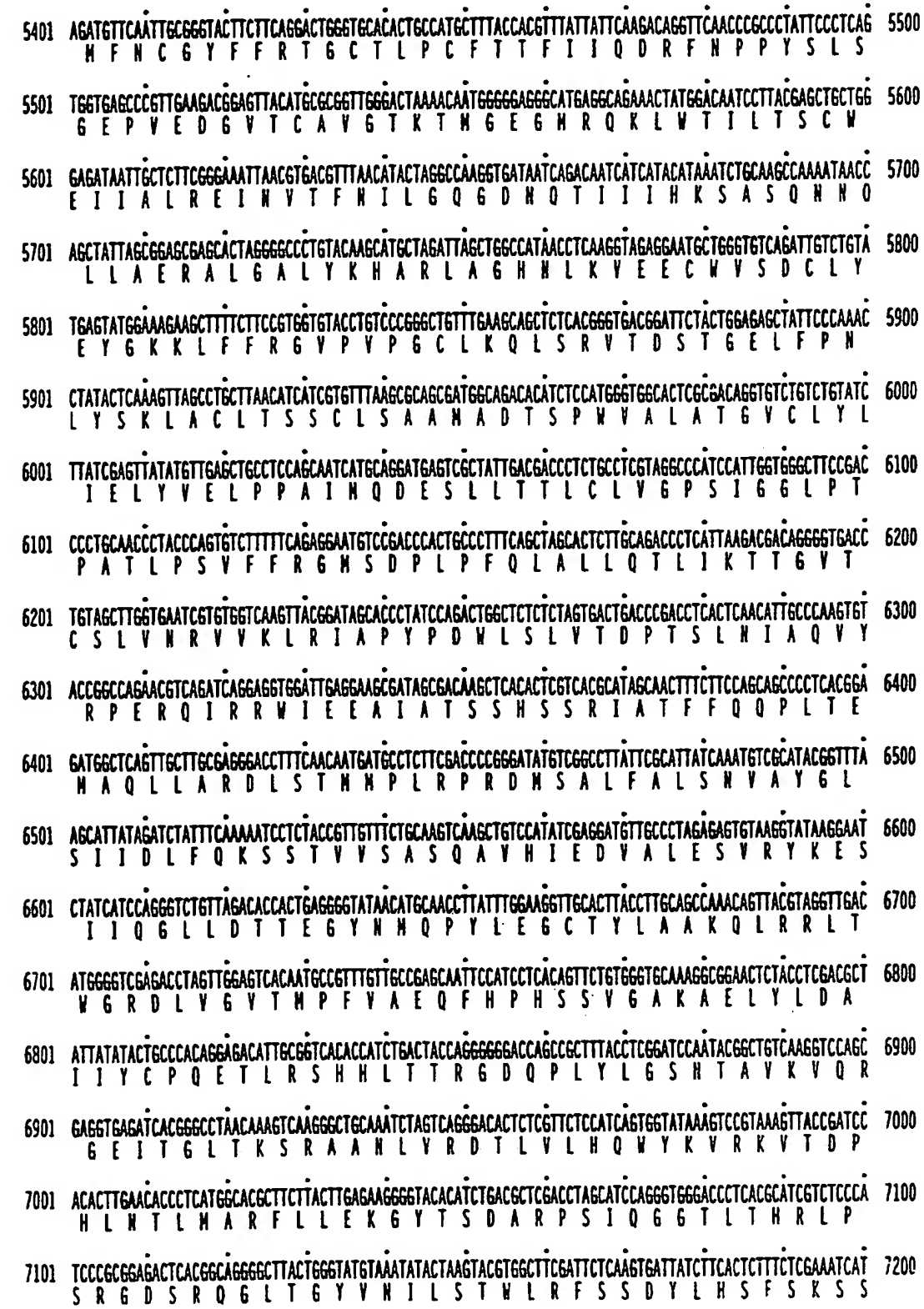
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FIG. 2(5)

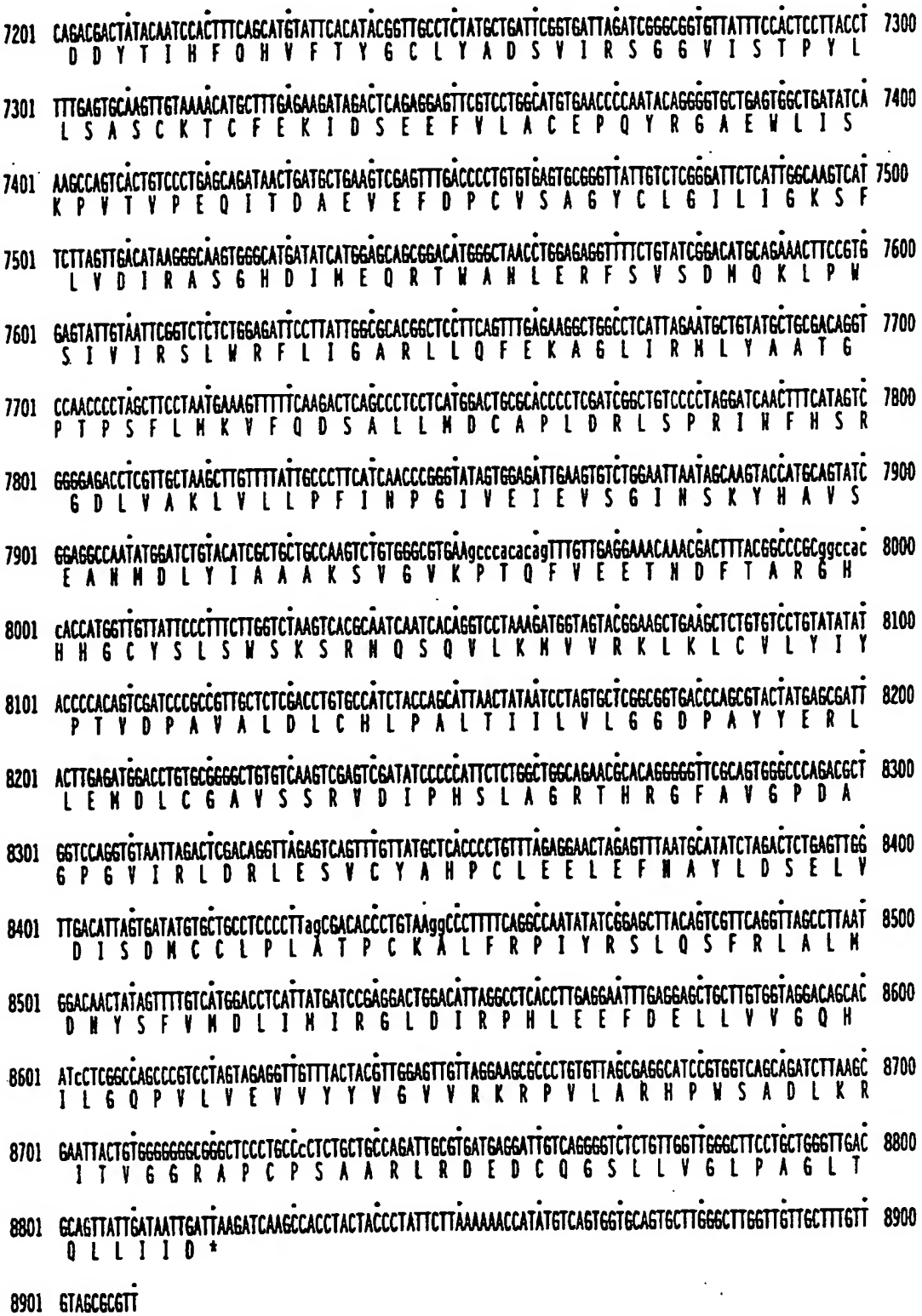
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FIG. 3a

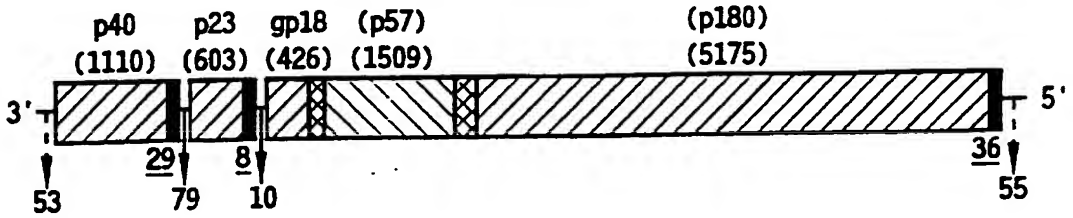
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FIG. 3b

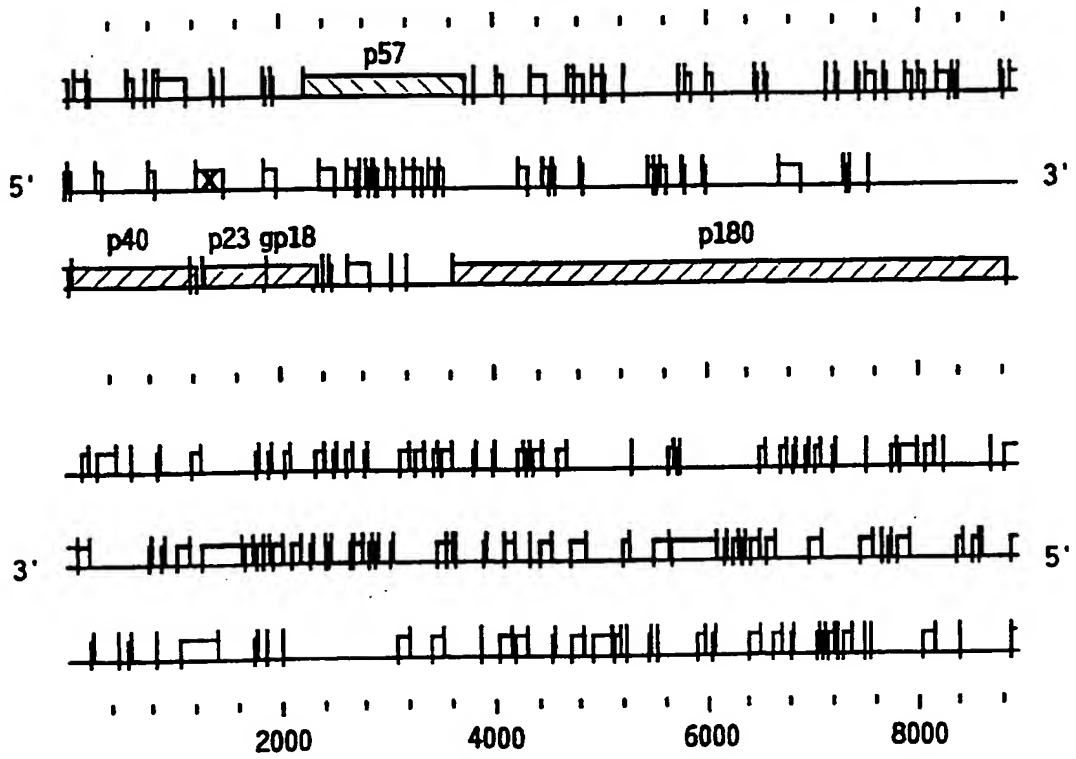
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FIG. 4(1)

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437 SeV  tais.yecav dnytsFigfk frkfiepqld eDLtiymkDK alsprkeawd svYpdsnly. ......yka. ...peseetr RliewFinde nfnpeeiiny vesgdwLkde efnisYslKE kEiKqeGRLF akMtykMRav qVlaEtLlak
433 NDV  aeis.hdiml reyksLsale fepcieydpv tnLsmflkDK aiahpndnwl asFrrnlls. ......edqk khvkeatstn RlieFLesn dfdpykemey lttleyLrdd dvavsYslKE kEvKvnGRIF akLtkkLRnc qVmaEgilad
450 MaV  sfps.qaely qhlveWyfve heplfstkii sDLsifikDr ltavnqecwd svFdrsvlg. ......ynpp vrfq....sk RvpeqFLgqa dfslnqilef aekleyLaps yrnfsFslKE kElni.GRtF gkLpyrvRnv qtlaEaLlad
501 RSV  typsllelte rdlivLsglr fyrefrlpkk vDLemiinDK aisppkmliw tsFprnympS hiqnylehek lkfsesdksr RvleyYLrdn kfnecdlync vvnqsyLnnp nhvvsLtgKE rElsv.GRMF amqpgmFRqv qllaEkMiae
    con  .......... .....V.... .......... .DL.....DK .......... .......... .......... .......... R....F1... .......... ......1... .....L..KE .E.K..GRFF ..m...1R.. .V..E.1...

                                                                                                          x   * A *x*                       x **   x             300
    151
RaV  yllplFdalt M...tdnlnk vfkkL..... .......... .......... .......... .......... .....idrvt ggglldysrv tyafhlDYeK WnnhqRlEst edvfsvldqv FGlkrvFsRt HefFqkawiY ysdrsdligl
VSV  yyvplFkglt M...addLts vikkM..... .......... .......... .......... .......... .....mdsss gggldyssv clanhiDYeK WnnhqRkEsn gpifrvmgqf LGypsLieRt HefFeksliY yngrpdimti
SYN  yYlkYfpmit M...sdnLls mvirL..... .......... .......... .......... .......... .....fdmtt ligdkgva.v tysmniDFsK WnqnmRertn agifdnldri LGfrsLisRt HsiFkacylY lcsgeyvpvi
BDV>>eVmpYlkths Mtmsstalth llnrl..... .......... .......... .......... .......... .....shtit kgd....... sfvinlDYss WcngfRpElq apicrqldqm FncgyFF.Rt gctlpcfttF iiqdrfnppy<<
MeV  glgkYFkdng Makdeqdltk alhtLavsgv pkdlkeshrg gpvlktysrs pvhtstrnvr aakgfigfpq virqdqdtdh penmeayetv safittDLkK YclnwRyEti slfaqrlnei YGlpsFFqWl HkrLetsvlY vsdphcppdl
SeV  glgeLFseng Mvkgeidllk rlttlsvsgv p.......rt dsvynnskss ekrnegmgnk nsggywdekk rsrhefkatd sst.dgyetl scfittDLkK YclnwRfEst alfggrcnei FGfktFFnWm HpvLerctiY vgdpycpvad
NDV  qlapFFggng viqdsisLtk s..tLamsql sfnsnkkrit dckervssnr nhdpksknrr .......... .......... ........rv atfittDLqK YclnwRyqti klfahainql mGlphFFeWi HlrLmdttmF vgdpfnppsd
MaV  glakaFpsnm Mvvtereqke allhqa.... .......... .svhhnsasi genaivr... .......... .......... .......... gasfvtDLeK YnlafRyEft rhfidycnrc YGvknLFdWm Hflipicymh vsdfysppkc
RSV  nllqFFpesl trygdleLqk ilelkagi.. ...snksnry ndnynnyisk .......... .......... .......... .......... .csiltDLsK FnqafRyEts cicsdvldel hGvqsLFsWl Hltiphvtii ctyrhappyi
Con  .l...F.... M......L.. ....L..... .......... .......... .......... .......... .......... .......... ......DL.k .....R.E.. .......... Fg....F.W. h..L.....Y ..........
```

FIG. 4(2)

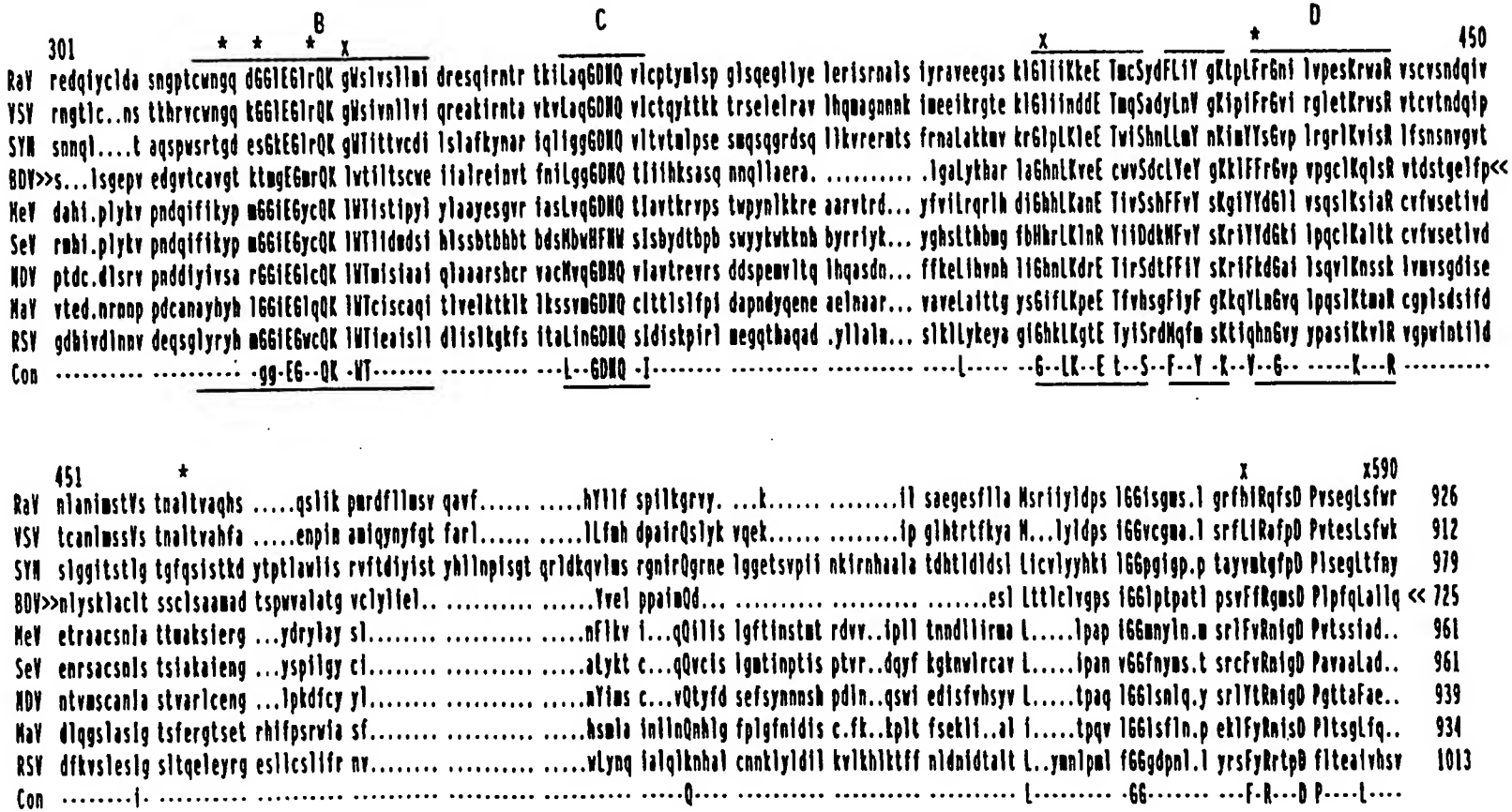

B
C
D
301
450
451
926
912
979
725
961
961
939
934
1013
RaV redqiyclda sngptcwngq dGGlEGlrQK gWslvsllml dresqirntr tkilaqGDNQ vlcptymlsp glsqeglIye lerisrnals iyraveegas klGliikkeE lmcSydFLiY gKtplFrGni lvpesKrwaR vscvsndqiv
VSV rngtlc..ns tkhrvcwngq kGGlEGlrQK gWsivnllvl qreakirnta vkvLaqGDNQ vlctqykttk trselelrav lhqnagnank imeeikrgte klGliinddE lmqSadyLnV gKipiFrGvi rgletKrvsR vtcvtndqip
SYN snnql....t aqspwsrtgd esGkEGlrQK gWlittvcdi lslafkynar iqliggGDNQ vltvtnlpse smqsqgrdsq llkvrernts frnalakkav krGlpLKleE TwiShnLLmY nKimYYsGvp lrgrlKvisR lfsnsnvgvt
BDV>>s...lsgepv edgvtcavgt ktmgEGmrQK lvtiltscve iialreinvt fnilggGDNQ tlilhksasq nnqllaera. ..... .lgaLykhar laGhnLKveE cwvSdcLYeY gKklFFrGvp vpgclKqlsR vtdstgelfp<<
MeV dahi.plykv pndqifikyp mGGiEGycQK lWTistipyl ylaayesgvr iasLvqGDNQ tlavtkrvps twpynlkkre aarvtrd... yfvilrqrlh diGhhLKanE TivSshFFvY sKgiYYdGll vsqslKsiaR cvfwsetivd
SeV rmhi.plykv pndqifikyp mGGiEGycQK lWTlidmdsi hlssbtbhbt bdsNbwHFNW slsbydtbpb swyykwkknh byrriyk... yghsltbbmg fbHhrLKlnR YiiDdkNFvY sKriYYdGki lpqclKaltk cvfwsetlvd
NDV ptdc.dlsrv pnddiyivsa rGGiEGlcQK lWTmisiaai qlaaarshcr vacNvqGDNQ vlavtrevrs ddspemvltq lhqasdn... ffkeLihvnh liGhnLKdrE TirSdtFFiY sKriFkdGai lsqvlKnssk lvmvsgdise
MaV vted.nrnnp pdcanayhyh lGGiEGlqQK lWTciscaqi tlvelktklk lkssvmGDNQ clttlslfpi dapndyqene aelnaar... vaveLaittg ysGifLKpeE TfvhsgFiyF gKkqYLnGvq lpqslKtmaR cgplsdsifd
RSV gdhivdlnnv deqsglyryh mGGiEGwcQK lWTieaisll dlislkgkfs itaLinGDNQ sldiskpirl negqthaqad .yllaln... slklLykeya giGhklLKgtE TyiSrdHqfm sKtiqhnGvy ypasiKkvlR vgpwintlld
Con .... -gg-EG--QK -WT..... ..... ---L--GDNQ -I..... ....L..... --G--LK--E t--S--F--Y -K--Y--G-- .....K---R .....
RaV nlanimstVs tnaltvaqhs .....qslik purdfllmsv qavf..... .....hYllf spilkgrvy. ...k..... .....il saegesflla Msriiyldps lGGisgms.l grfhiRqfsD Pvsegisfwr
VSV tcanlmssVs tnaltvahfa .....enpln anlqynyfgt farl..... .....lLfmh dpairQslyk vqek..... .....ip glhtrtfkya M...lyldps lGGvcgma.l srfLiRafpD PvtesLsfwk
SYN slggitstlg tgfqsistkd ytptlawlis rvftdiyist yhllnpisgt qrldkqvlms rgnirQgrne lggetsvpli nkirnhaala tdhtldldsl Licvlyyhki lGGpgigp.p tayvnkgfpD PlsegLtfny
BDV>>nlysklaclt ssclsaamad tspwvalatg vclyliel.. ....Yvel ppaimQd... ....esl Lttlclvgps lGGlptpatl psvFfRgmsD PlpfqLallq << 725
MeV etraacsnla ttnaksierg ...ydrylay sl..... ....nflkv i...qQilis lgftinstnt rdvv..ipll tnndllirma L.....lpap lGGmnyln.m srlFvRnigD Pvtssiad..
SeV enrsacsnls tsiakaieng ...yspilgy cl..... ....alykt c...qQvcls lgntinptis ptvr..dqyf kgknvlrcav L.....ipan vGGfnyns.t srcFvRnigD PavaaLad..
NDV ntvnscanla stvarlceng ...lpkdfcy yl..... ....nYins c...vQtyfd sefsynnnsh pdln..qswi edisfvhsyv L.....tpaq lGGlsnlq.y srlYtRnigD PgttaFae..
MaV dlqgslaslg tsfergtset rhlfpsrvla sf..... ....hsnla inllnQnhlg fplgfnidis c.fk..kplt fsekli..al i.....tpqv lGGlsfln.p eklFyRnisD PltsgLfq..
RSV dfkvslesig sltqeleyrg esllcslifr nv..... ....wLynq lalqlknhal cnnklyldil kvlthlttff nldnidtalt L..ynnlpnl fGGgdpnl.l yrsFyRrtpD fltealvhsv
Con .....|. ....Q---- .... L..... .GG..... ---F-R---D P----L----

FIG. 5a

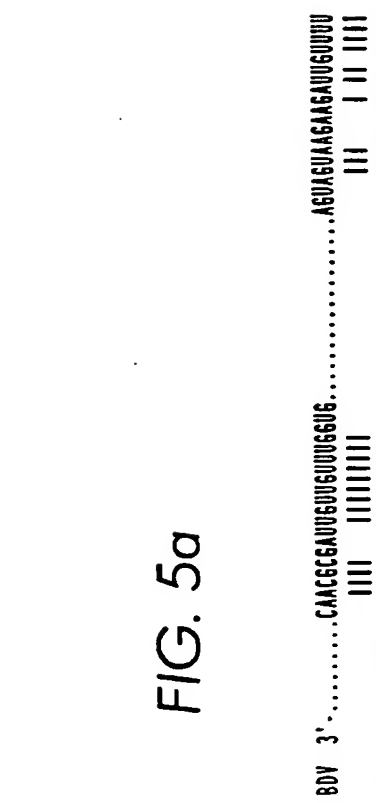

BDV 3'-.....CAACGCGAUUGUGUUUGGUG.....AGUAGUAAGAAGAUUGUUUU
RSV 3'-UGCGCUUUUUUUACGC..AUGUGUUUG.....AACGUAUUU.GGUUUUUUU
EboV 3'-.....ACCCGUGUGUUUUUCUUUCGUCUAAAAAUCCUAG.AAAACACACG...UUAUUGAU
MaV 3'-.....UCUGUGUGUUUU.GUUCU.CUACUACU.....AAAACACAUAG.UAUAUUUAU
NDV 3'-.....UGGUUU..GU.CU.CUUAGGCAUUCA.AUGCUAUUUUCCG.CUUCCUCGU
HeV 3'-.....UGGUUU..GUUUC...AACCCAUUCCUAUCAAGUUUA.....GUUACUAGU
RaV 3'-.....UGCGAUUGGUCUA.GUUUCUUUUUU..GUCUGUAAC.AGUUAAC...GUUUCGUUU
VSV 3'-.....UGCUUCU.GUUUUUUUGGUAAGAAUGUUA..AUAAACGGGA.UCUCCCUU

FIG. 5b

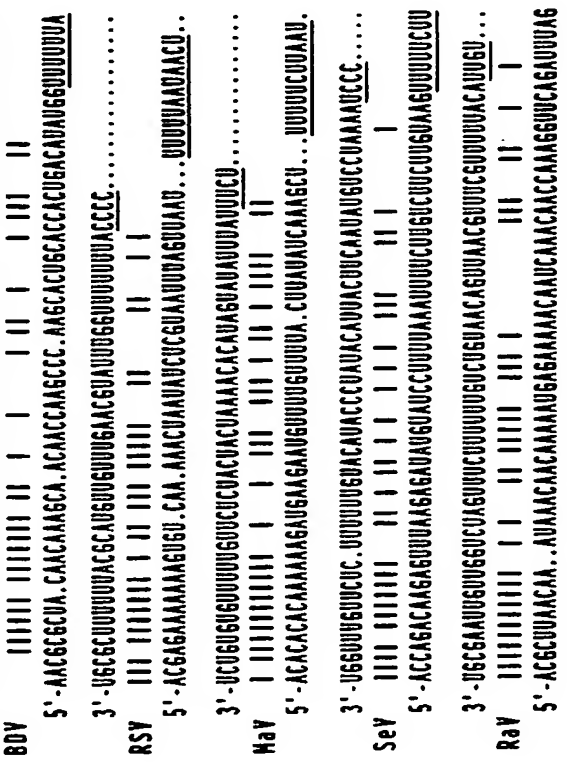

3'-CAACGCGAUUGUUUGGUGAGUAGUAAGAAGAUUGUUUUACUUGUGUGCGUUACGGUGGGUUC
BDV
5'-AACGCGCUA.CAACAAAGCA.ACAACCAAGCCC.AAGCACUGCACCACUGACAUAUGGUUUUUUA
3'-UGCGCUUUUUUUACGCAUGUGUUUGAACGUAUUUGGUUUUUUUACCCC.....
RSV
5'-ACGAGAAAAAAAGUGU.CAA.AAACUAAUAUCUCGUAAUUUAGUUAAG...UUUUUAAUAACU..
3'-UCUGUGUGUUUUUGUUCUCUACUACUAAAAACACAUAGUAUAUUUAUUUCU.....
MaV
5'-ACACACACAAAAAAAGAUGAAGAAUGUUUUGUUUUA.CUUAUAUCAAAGCU...UUUUUCUUAAU.
3'-UGGUUUGUUCUC..UUUUUUGUACAUACCCUAUACAUUACUUCAAUAUGUCCUAAAAUCCC.....
SeV
5'-ACCAGACAAGAGUUUAAGAUAUGUAUCCUUUUAAAUUUUCUUGUCUUCUUGUAAGUUUUUCUU
3'-UGCGAAUUGUUGGUCUAGUUUCUUUUUUUGUCUGUAACAGUUAACGUUUCGUUUUUACAUUGU...
RaV
5'-ACGCUUAACAA..AUAAACAACAAAAAUGAGAAAAACAAUCAAACACCAAAGGUUCAGAUUUAG

FIG. 6a

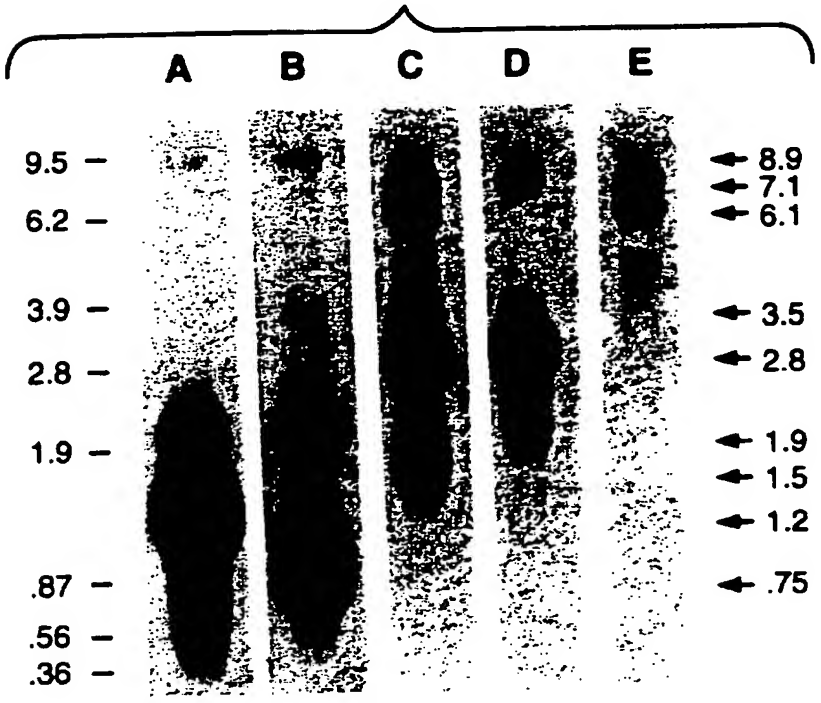
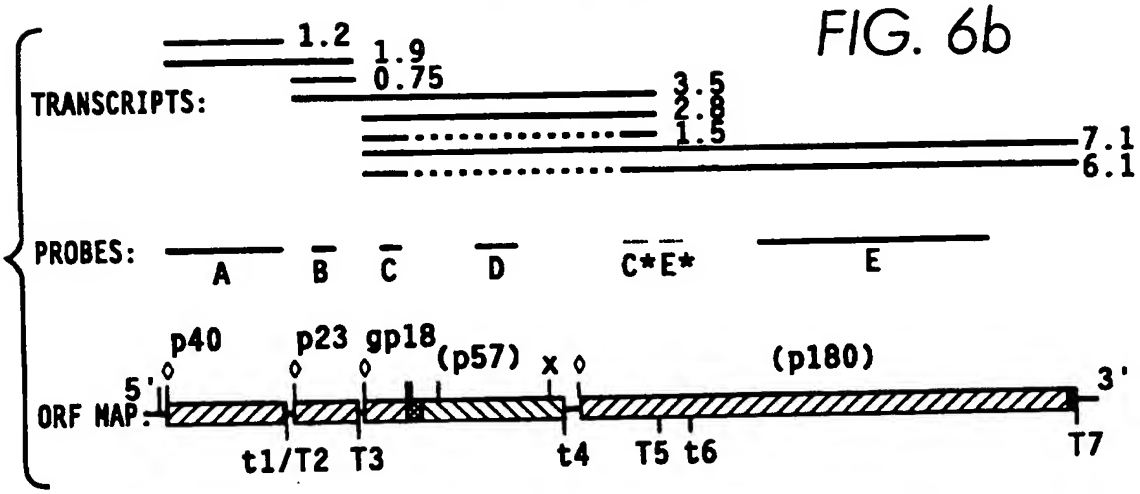


FIG. 6b



(t1)

p40 5'-GUcU AAa cua aaa aaC AAU GAA caa acc aau aaaaaa CCA AAU GCG (T2)

P23 5'-CGAC AAC GGA UGA AUG GGA CAU cau acc aua aaaaaa UCG AAU CAC (T3)

gp18 5'-UUAG CUG CAA UAC UGA CUC CAC UCC UGG ACU GAU UGA CCU

P57 5'-GGCA GGA AUA AAC CGU ACC GAC cag Ucu cuu aaaaa CCC UCU CCU (t4)

5'-CUGA AUU AUG CCC GCA ACU AGA cag cuu acu aaaaaaACUG CUC CAA (T5)

5'-UACA CCU GCU CCC AGG CUG UGA caa auc cau aaaaaa UGC GAG AGA (t6)

P180 5'-UUAA GAU CAA GCC ACC UAC UAC ccu auu cuu aaaaaa CCA UAU GUC (T7)

FIG. 6c

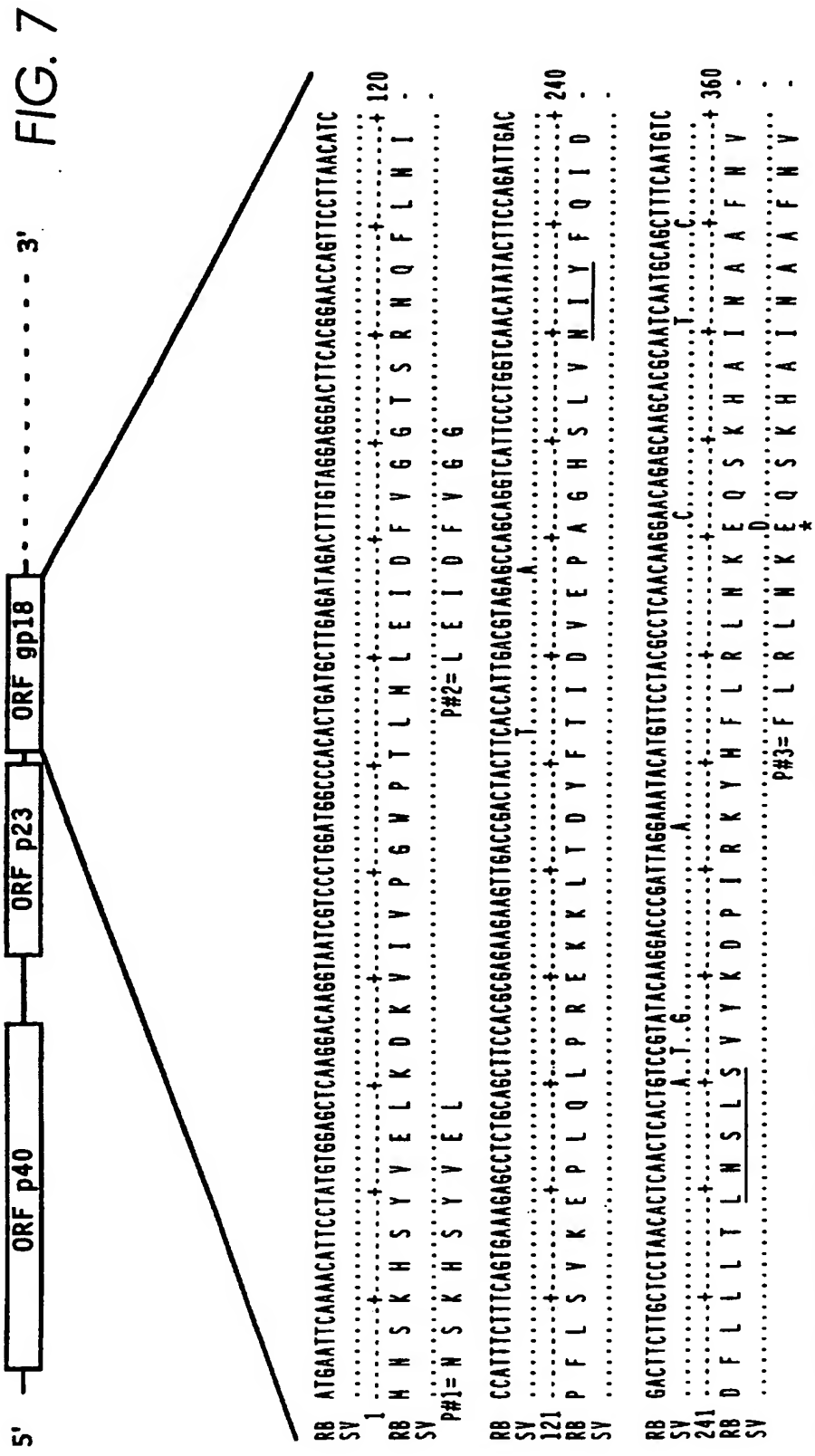

5'
ORF p40
ORF p23
ORF gp18
3'
RB ATGAATTCAAAACATTCCTATGTGGAGCTCAAGGACAAGGTAATCGTCCCTGGATGCCCACACTGATGCTTGAGATAGACTTTGTAGGAGGGACTTCACGGAACCAGTTCCTTAACATC
SV
1
RB M N S K H S Y Y V E L K D K V I V P G W P T L M L E E I D F V G G T S R N Q F L N I -
SV
P#1= N S K H S Y Y V E L P#2= L E E I D F V G G
RB CCATTTCTTTCAGTGAAAGAGCCTCTGCAGCTCCACGCGAGAAGAAGTTGACCGACTACTTCACCATTGACGTAGAGCCAGCAGGTCATTCCCTGGTCAACATATACTTCCAGATTGAC
SV
121
RB P F L S V K E P L Q L P R E K K L T D Y F T I D V E P A G H S L V N I Y F Q I D -
SV
RB GACTTCTTGCTCCTAACACTCAACTCACTGTCCGTATACAAGGACCCGATTAGGAAATACATGTTCCTACGCCTCAACAAGGAACAGAGCAGCAAGCACGCAATCAATGCAGCTTTCAATGTC
SV
241
RB D F L L L T L N S L S V Y Y K D P I R K Y H F L R L N K E Q S K H A I N A A F N V -
SV
P#3= F L R L N K E Q S K H A I N A A F N V -
RB TTCTCTTATCGGCTTCGGAACATTGGTGTTGGTCCTCTCGGCCCAGACATTCGATCTTCAGGGCCTTAG
SV
361
RB F S Y R L R H I G V G P L G P D I R S S G P * -
SV
F S Y R L R H I G V G P L G

FIG. 7

FIG.8

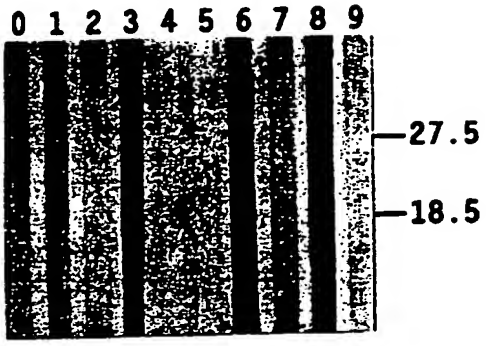


FIG.9

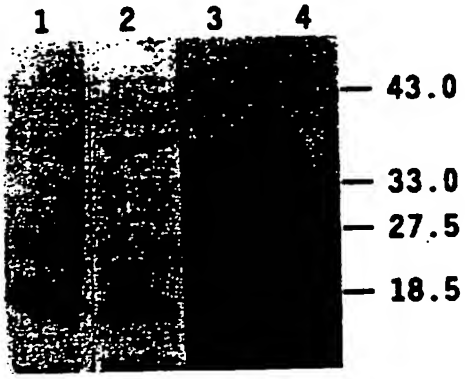


FIG.10A

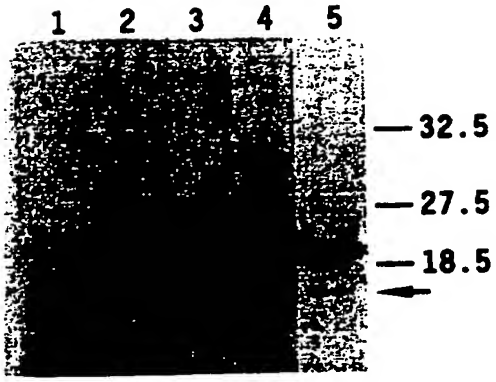
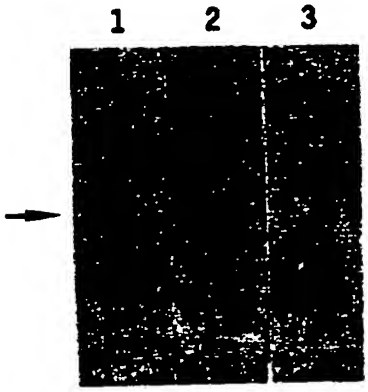


FIG.10B



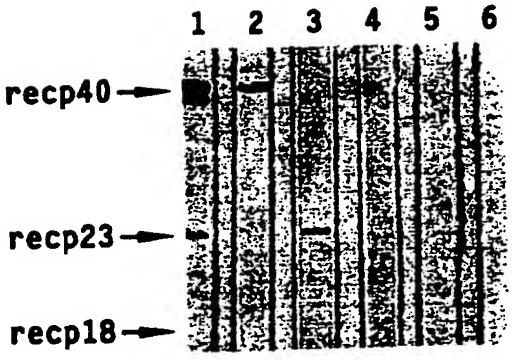


FIG.11A

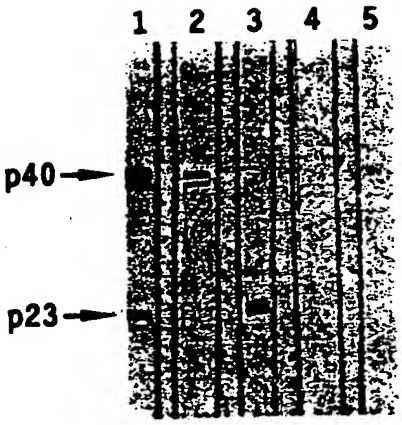


FIG.11B

FIG. 12

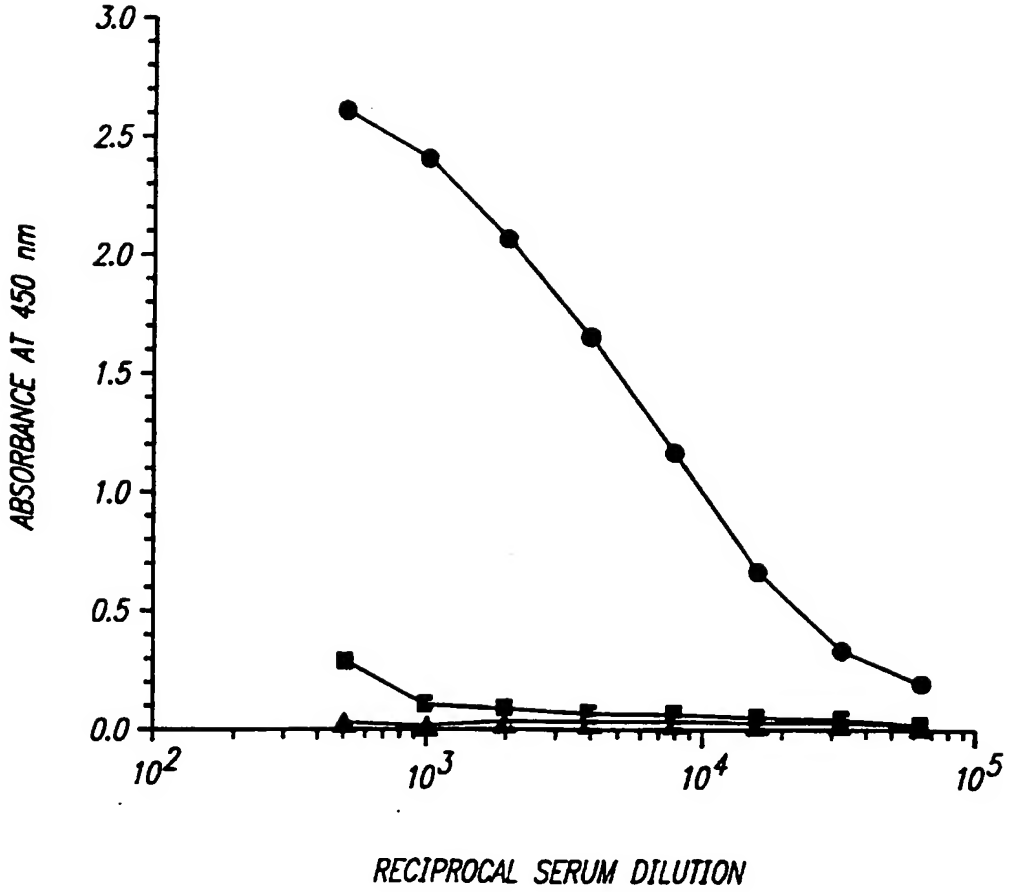

3.0
2.5
2.0
1.5
1.0
0.5
0.0
ABSORBANCE AT 450 nm
10^2
10^3
10^4
10^5
RECIPROCAL SERUM DILUTION

FIG. 13A

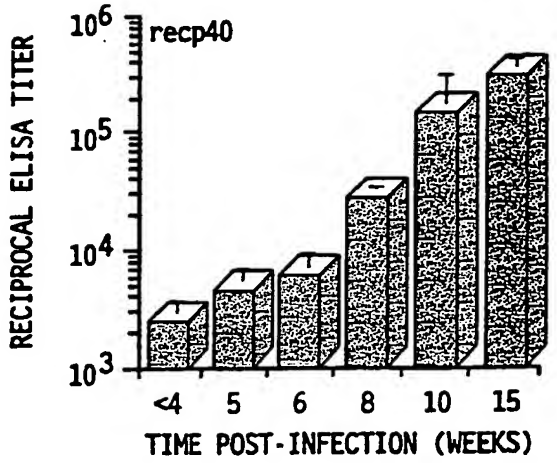


FIG. 13B

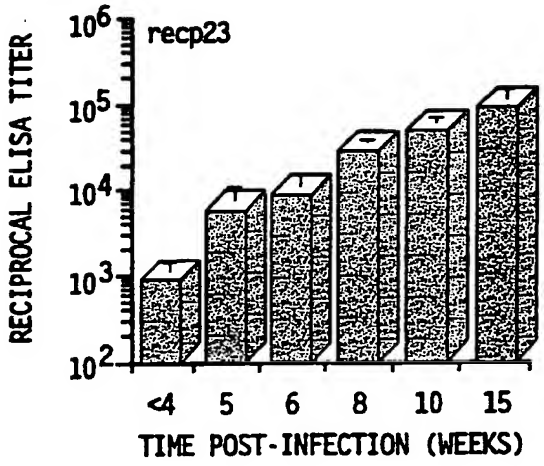


FIG. 13C

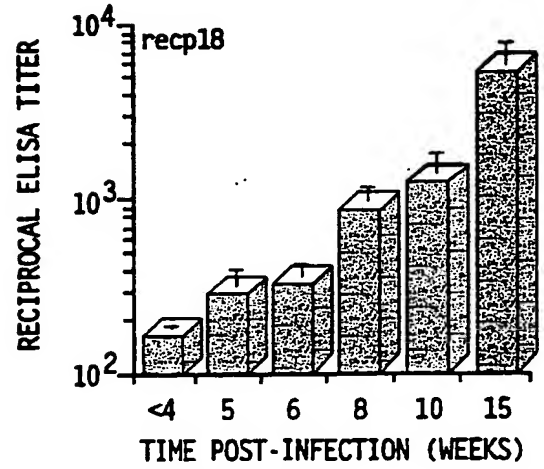


FIG. 14A

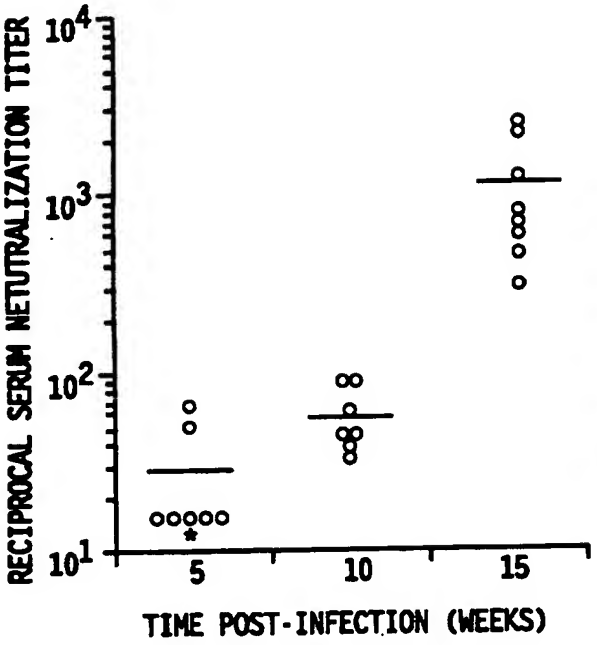


FIG. 14C

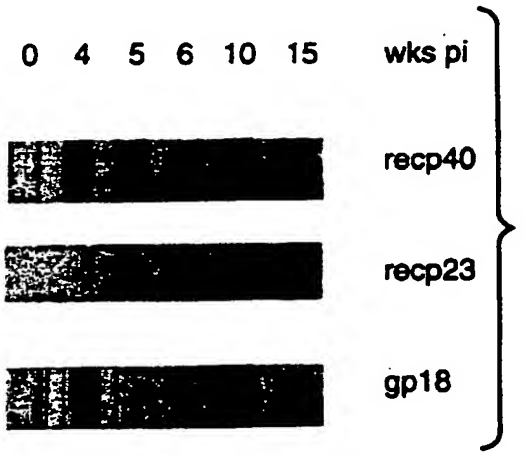


FIG. 14B

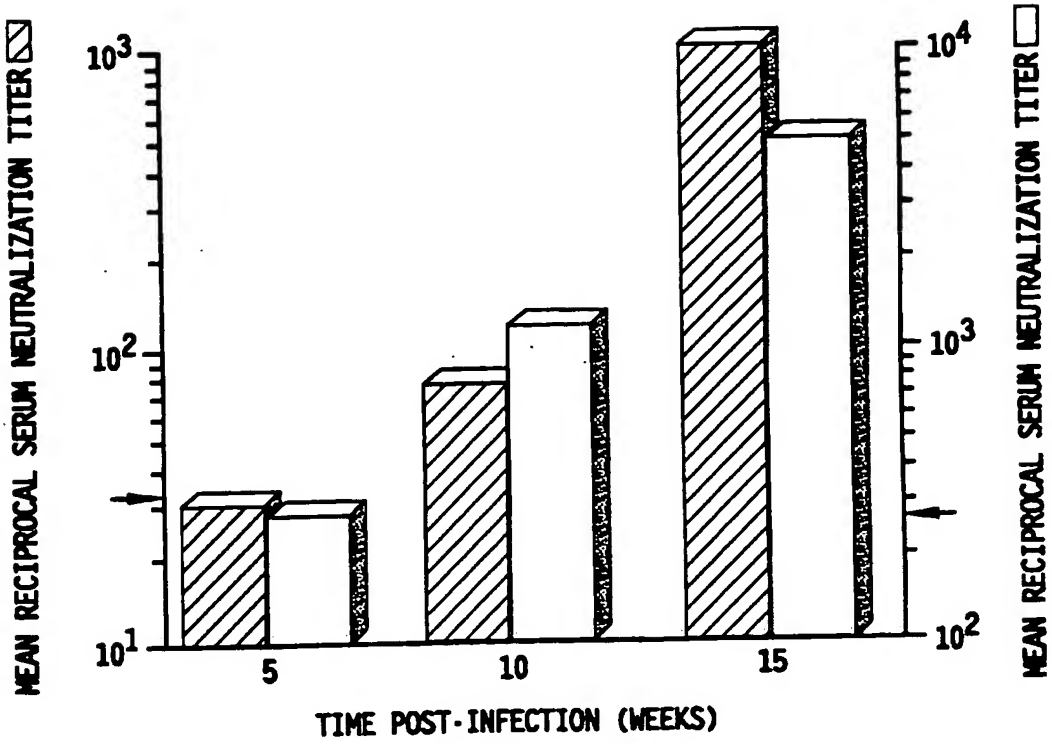
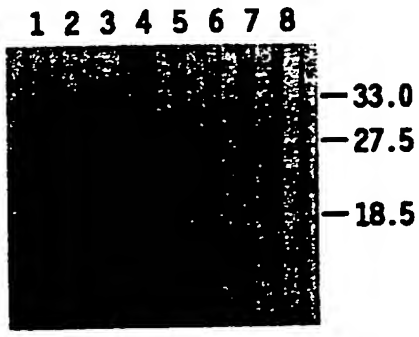


FIG. 15A

FIG. 15B



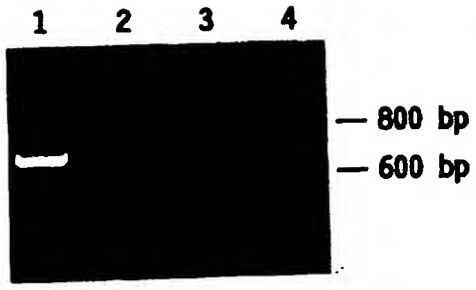


FIG. 17A

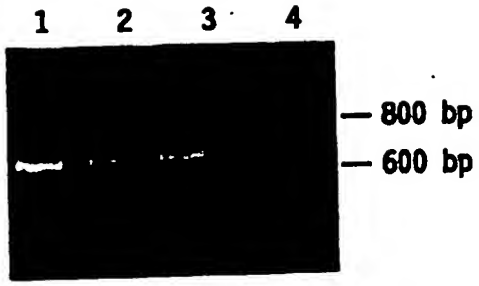
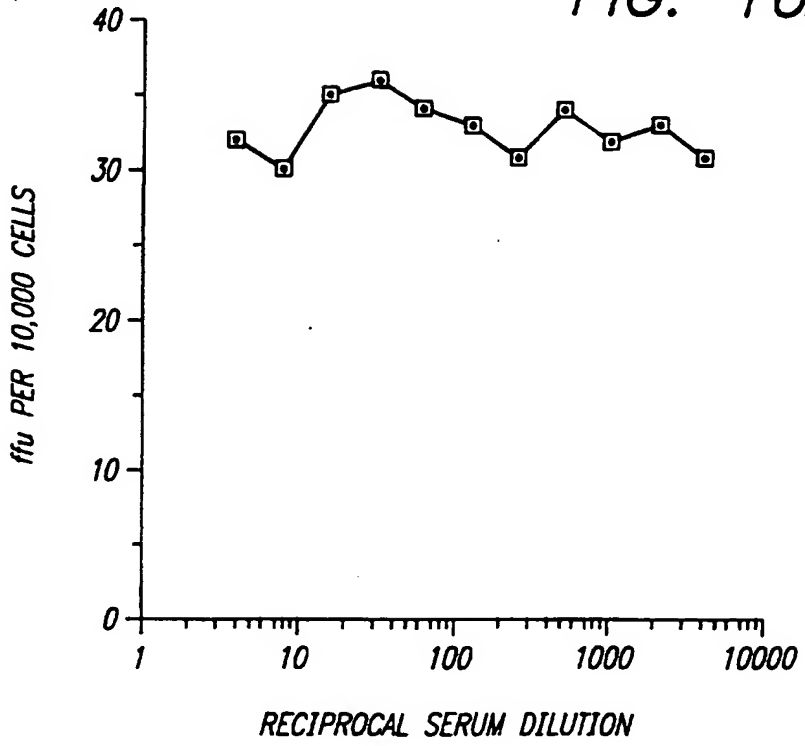


FIG. 17B

FIG. 16A



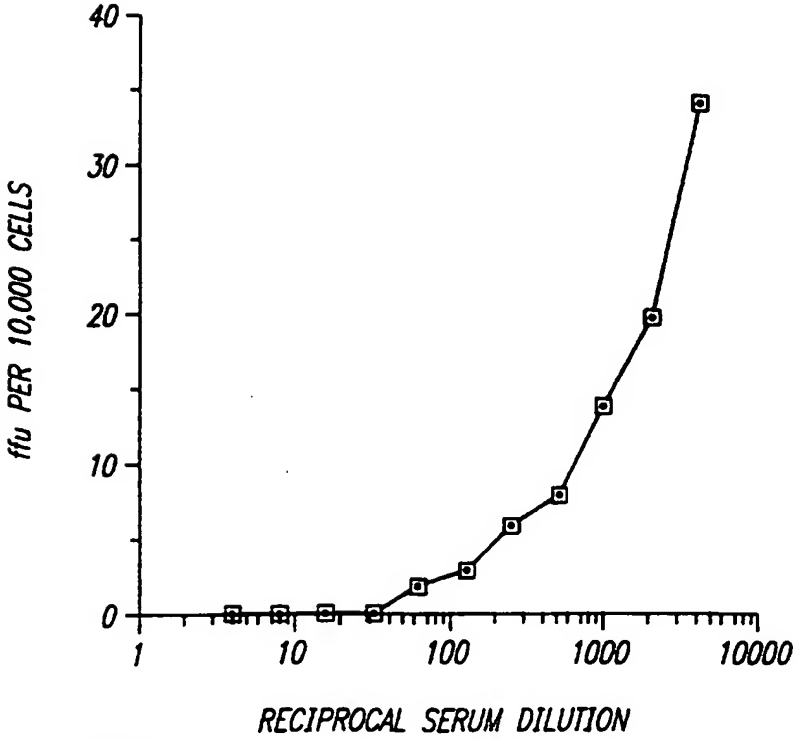
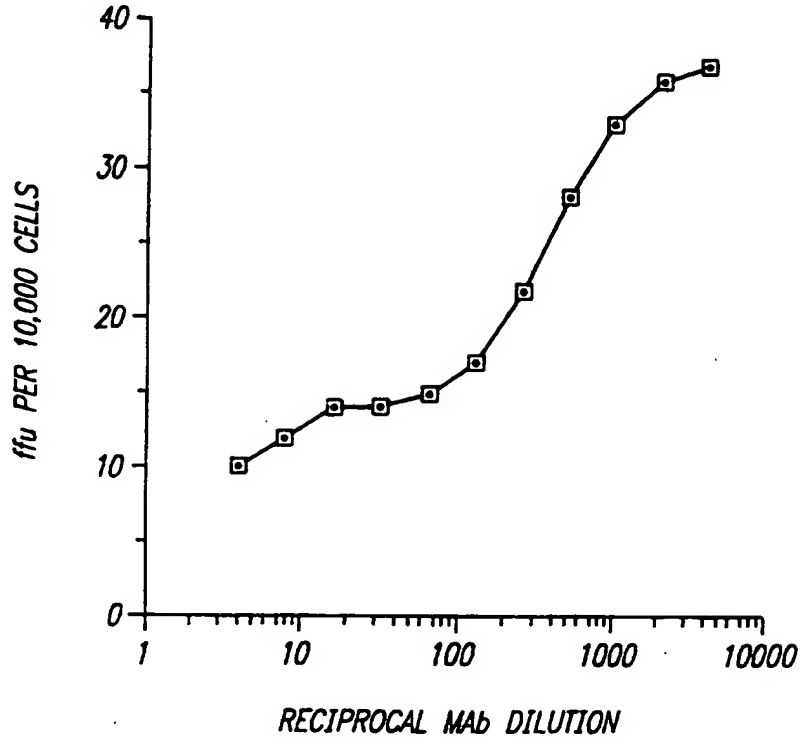


FIG. 16B

FIG. 16C



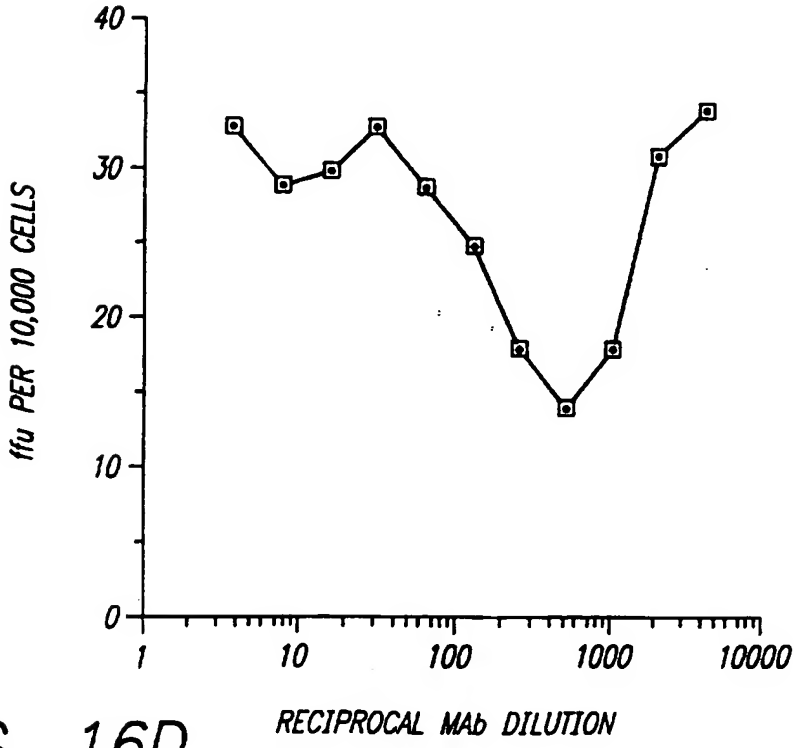


FIG. 16D

FIG. 18(1)

POLYMERASE NUCLEOTIDE SEQUENCE

I-2

```
          ATGTCATTTCATGCGAG\ /CCTCCTTCGCGAGGAGGAGACGCCTCGGCCGGTGGCAGGA
2393      ------+---------+ V ------+---------+---------+---------+---  3743
          TACAGTAAAGTACGCTC   GGAGGAAGCGCTCCTCCTCTGCGGAGCCGGCCACCGTCCT

          ATAAACCGTACCGACCAGTCTCTTAAAAACCCTCTCCTCGGAACAGAGGTCTCTTTCTGC
3744      ------+---------+---------+---------+---------+---------+---  3803
          TATTTGGCATGGCTGGTCAGAGAATTTTTGGGAGAGGAGCCTTGTCTCCAGAGAAAGACG

          CTTAAGTCGAGCTCACTCCCCCATCATGTACGAGCACTAGGCCAGATTAAAGCAAGGAAC
3804      ------+---------+---------+---------+---------+---------+---  3863
          GAATTCAGCTCGAGTGAGGGGGTAGTACATGCTCGTGATCCGGTCTAATTTCGTTCCTTG

          CTGGCATCCTGTGACTATTACTTGCTATTCCGCCAAGTTGTATTGCCCCCTGAAGTATAT
3864      ------+---------+---------+---------+---------+---------+---  3923
          GACCGTAGGACACTGATAATGAACGATAAGGCGGTTCAACATAACGGGGGACTTCATATA

          CCCATTGGTGTTCTAATAAGAGCTGCGGAGGCTATACTAACAGTTATAGTATCAGCTTGG
3924      ------+---------+---------+---------+---------+---------+---  3983
          GGGTAACCACAAGATTATTCTCGACGCCTCCGATATGATTGTCAATATCATAGTCGAACC

          AAGCTGGATCATATGACGAAGACCCTATACTCCTCTGTGAGATATGCACTCACCAATCCC
3984      ------+---------+---------+---------+---------+---------+---  4043
          TTCGACCTAGTATACTGCTTCTGGGATATGAGGAGACACTCTATACGTGAGTGGTTAGGG

          CGGGTCCGAGCCCAACTTGAGCTTCACATTGCCTACCAGCGCATAGTGGGTCAGGTCTCG
4044      ------+---------+---------+---------+---------+---------+---  4103
          GCCCAGGCTCGGGTTGAACTCGAAGTGTAACGGATGGTCGCGTATCACCCAGTCCAGAGC

          TACAGCCGGGAGGCAGACATAGGGCCAAAAAGGCTTGGGAATATGTCATTGCAATTCATC
4104      ------+---------+---------+---------+---------+---------+---  4163
          ATGTCGGCCCTCCGTCTGTATCCCGGTTTTTCCGAACCCTTATACAGTAACGTTAAGTAG

          CAATCTCTCGTTATTGCCACCATAGACACGACAAGCTGCCTAATGACCTACAACCACTTT
4164      ------+---------+---------+---------+---------+---------+---  4223
          GTTAGAGAGCAATAACGGTGGTATCTGTGCTGTTCGACGGATTACTGGATGTTGGTGAAA

          CTTGCTGCAGCAGACACAGCCAAGAGCAGATGCCATCTCCTAATCGCCTCAGTGGTCCAG
4224      ------+---------+---------+---------+---------+---------+---  4283
          GAACGACGTCGTCTGTGTCGGTTCTCGTCTACGGTAGAGGATTAGCGGAGTCACCAGGTC

          GGGGCCCTTTGGGAACAAGGGTCATTTCTTGATCATATAATCAACATGATCGACATAATT
4284      ------+---------+---------+---------+---------+---------+---  4343
          CCCCGGGAAACCCTTGTTCCCAGTAAAGAACTAGTATATTAGTTGTACTAGCTGTATTAA

          GACTCAATCAACCTCCCCCATGATGATTACTTCACAATTATTAAGTCTATCTTTCCCTAC
4344      ------+---------+---------+---------+---------+---------+---  4403
          CTGAGTTAGTTGGAGGGGGTACTACTAATGAAGTGTTAATAATTCAGATAGAAAGGGATG

          TCCCAAGGGCTTGTTATGGGGAGGCATAATGTATCAGTCTCCTCTGATTTCGCGTCCGTA
4404      ------+---------+---------+---------+---------+---------+---  4463
          AGGGTTCCCGAACAATACCCCTCCGTATTACATAGTCAGAGGAGACTAAAGCGCAGGCAT

          TTTGCCATTCCTGAATTATGCCCGCAACTAGACAGCTTACTAAAAAAACTGCTCCAACTT
4464      ------+---------+---------+---------+---------+---------+---  4523
          AAACGGTAAGGACTTAATACGGGCGTTGATCTGTCGAATGATTTTTTTGACGAGGTTGAA

          GACCCCGTTCTCCTCCTCATGGTCTCTTCGGTGCAGAAGTCATGGTACTTCCCTGAGATC
4524      ------+---------+---------+---------+---------+---------+---  4583
          CTGGGGCAAGAGGAGGAGTACCAGAGAAGCCACGTCTTCAGTACCATGAAGGGACTCTAG
```

FIG. 18(2)

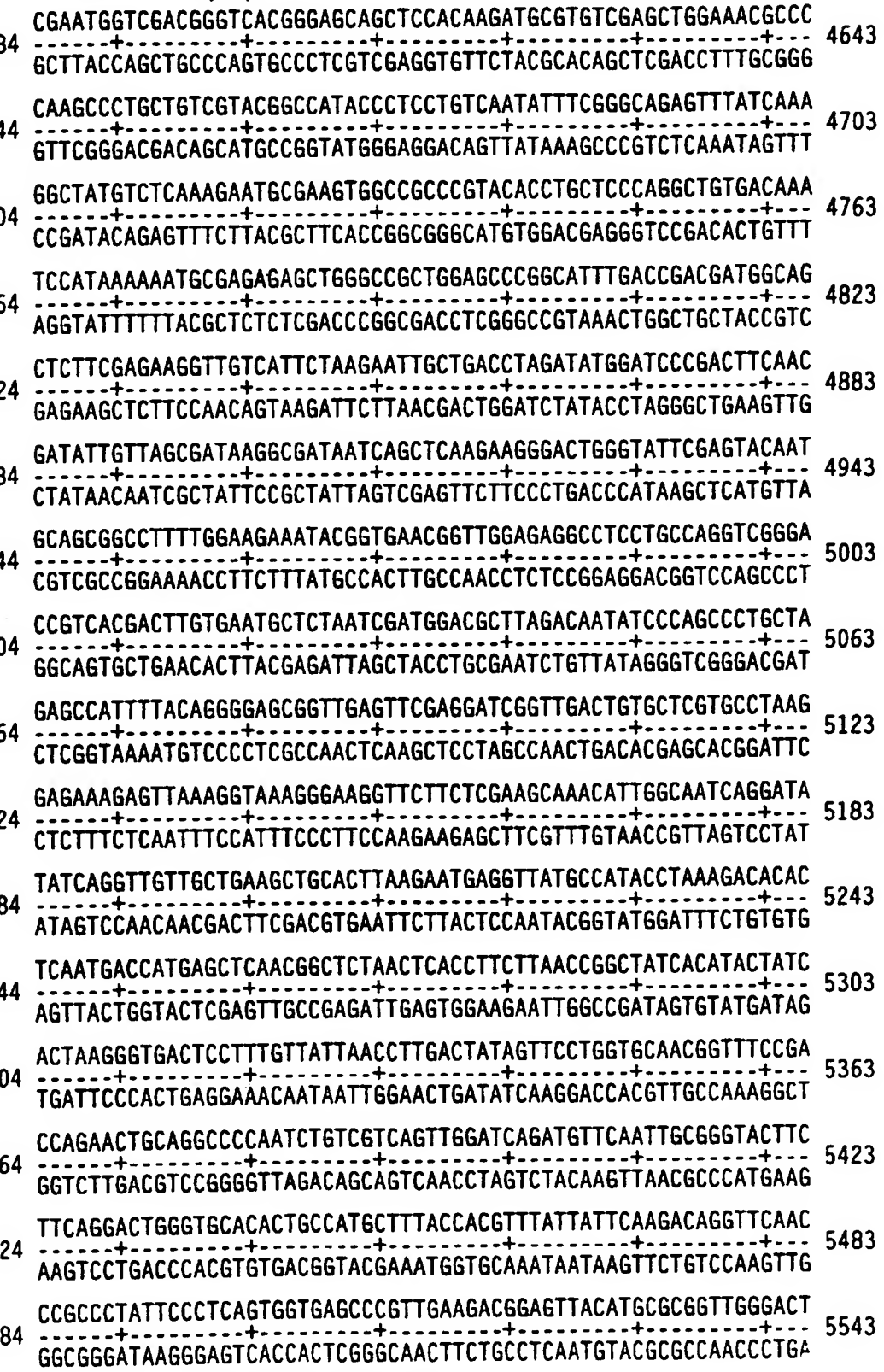
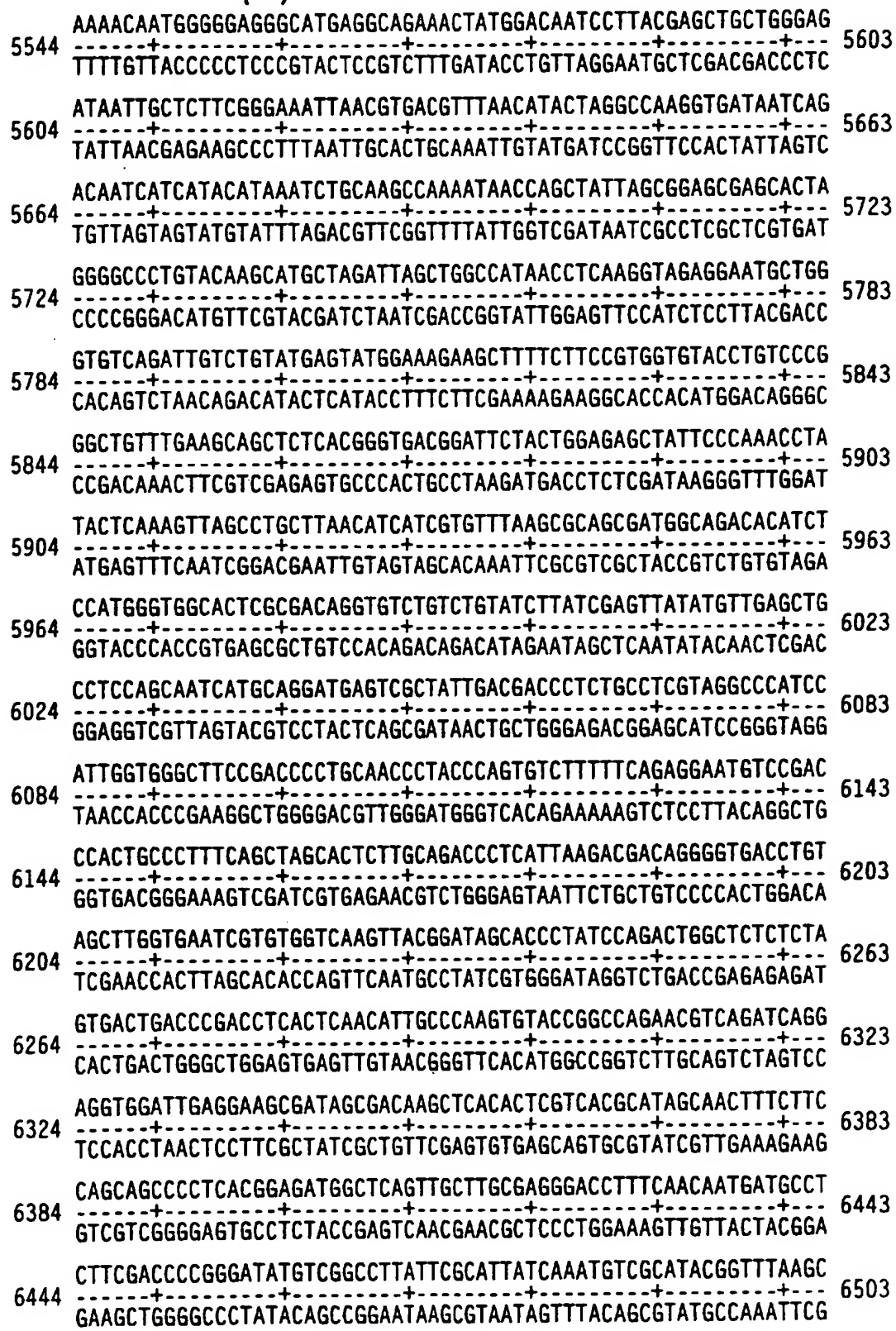

4584 CGAATGGTCGACGGGTCACGGGAGCAGCTCCACAAGATGCGTGTCGAGCTGGAAACGCCC GCTTACCAGCTGCCCAGTGCCCTCGTCGAGGTGTTCTACGCACAGCTCGACCTTTGCGGG 4643
4644 CAAGCCCTGCTGTCGTACGGCCATACCCTCCTGTCAATATTTCGGGCAGAGTTTATCAAA GTTCGGGACGACAGCATGCCGGTATGGGAGGACAGTTATAAAGCCCGTCTCAAATAGTTT 4703
4704 GGCTATGTCTCAAAGAATGCGAAGTGGCCGCCCGTACACCTGCTCCCAGGCTGTGACAAA CCGATACAGAGTTTCTTACGCTTCACCGGCGGGCATGTGGACGAGGGTCCGACACTGTTT 4763
4764 TCCATAAAAAATGCGAGAGAGCTGGGCCGCTGGAGCCCGGCATTTGACCGACGATGGCAG AGGTATTTTTTACGCTCTCTCGACCCGGCGACCTCGGGCCGTAAACTGGCTGCTACCGTC 4823
4824 CTCTTCGAGAAGGTTGTCATTCTAAGAATTGCTGACCTAGATATGGATCCCGACTTCAAC GAGAAGCTCTTCCAACAGTAAGATTCTTAACGACTGGATCTATACCTAGGGCTGAAGTTG 4883
4884 GATATTGTTAGCGATAAGGCGATAATCAGCTCAAGAAGGGACTGGGTATTCGAGTACAAT CTATAACAATCGCTATTCCGCTATTAGTCGAGTTCTTCCCTGACCCATAAGCTCATGTTA 4943
4944 GCAGCGGCCTTTTGGAAGAAATACGGTGAACGGTTGGAGAGGCCTCCTGCCAGGTCGGGA CGTCGCCGGAAAACCTTCTTTATGCCACTTGCCAACCTCTCCGGAGGACGGTCCAGCCCT 5003
5004 CCGTCACGACTTGTGAATGCTCTAATCGATGGACGCTTAGACAATATCCCAGCCCTGCTA GGCAGTGCTGAACACTTACGAGATTAGCTACCTGCGAATCTGTTATAGGGTCGGGACGAT 5063
5064 GAGCCATTTTACAGGGGAGCGGTTGAGTTCGAGGATCGGTTGACTGTGCTCGTGCCTAAG CTCGGTAAAATGTCCCCTCGCCAACTCAAGCTCCTAGCCAACTGACACGAGCACGGATTC 5123
5124 GAGAAAGAGTTAAAGGTAAAGGGAAGGTTCTTCTCGAAGCAAACATTGGCAATCAGGATA CTCTTTCTCAATTTCCATTTCCCTTCCAAGAAGAGCTTCGTTTGTAACCGTTAGTCCTAT 5183
5184 TATCAGGTTGTTGCTGAAGCTGCACTTAAGAATGAGGTTATGCCATACCTAAAGACACAC ATAGTCCAACAACGACTTCGACGTGAATTCTTACTCCAATACGGTATGGATTTCTGTGTG 5243
5244 TCAATGACCATGAGCTCAACGGCTCTAACTCACCTTCTTAACCGGCTATCACATACTATC AGTTACTGGTACTCGAGTTGCCGAGATTGAGTGGAAGAATTGGCCGATAGTGTATGATAG 5303
5304 ACTAAGGGTGACTCCTTTGTTATTAACCTTGACTATAGTTCCTGGTGCAACGGTTTCCGA TGATTCCCACTGAGGAAACAATAATTGGAACTGATATCAAGGACCACGTTGCCAAAGGCT 5363
5364 CCAGAACTGCAGGCCCCAATCTGTCGTCAGTTGGATCAGATGTTCAATTGCGGGTACTTC GGTCTTGACGTCCGGGGTTAGACAGCAGTCAACCTAGTCTACAAGTTAACGCCCATGAAG 5423
5424 TTCAGGACTGGGTGCACACTGCCATGCTTTACCACGTTTATTATTCAAGACAGGTTCAAC AAGTCCTGACCCACGTGTGACGGTACGAAATGGTGCAAATAATAAGTTCTGTCCAAGTTG 5483
5484 CCGCCCTATTCCCTCAGTGGTGAGCCCGTTGAAGACGGAGTTACATGCGCGGTTGGGACT GGCGGGATAAGGGAGTCACCACTCGGGCAACTTCTGCCTCAATGTACGCGCCAACCCTGA 5543

FIG. 18(3)


AAAACAATGGGGGAGGGCATGAGGCAGAAACTATGGACAATCCTTACGAGCTGCTGGGAG
5544 5603
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ATAATTGCTCTTCGGGAAATTAACGTGACGTTTAACATACTAGGCCAAGGTGATAATCAG
5604 5663
TATTAACGAGAAGCCCTTTAATTGCACTGCAAATTGTATGATCCGGTTCCACTATTAGTC
ACAATCATCATACATAAATCTGCAAGCCAAAATAACCAGCTATTAGCGGAGCGAGCACTA
5664 5723
TGTTAGTAGTATGTATTTAGACGTTCGGTTTTATTGGTCGATAATCGCCTCGCTCGTGAT
GGGGCCCTGTACAAGCATGCTAGATTAGCTGGCCATAACCTCAAGGTAGAGGAATGCTGG
5724 5783
CCCCGGGACATGTTCGTACGATCTAATCGACCGGTATTGGAGTTCCATCTCCTTACGACC
GTGTCAGATTGTCTGTATGAGTATGGAAAGAAGCTTTTCTTCCGTGGTGTACCTGTCCCG
5784 5843
CACAGTCTAACAGACATACTCATACCTTTCTTCGAAAAGAAGGCACCACATGGACAGGGC
GGCTGTTTGAAGCAGCTCTCACGGGTGACGGATTCTACTGGAGAGCTATTCCCAAACCTA
5844 5903
CCGACAAACTTCGTCGAGAGTGCCCACTGCCTAAGATGACCTCTCGATAAGGGTTTGGAT
TACTCAAAGTTAGCCTGCTTAACATCATCGTGTTTAAGCGCAGCGATGGCAGACACATCT
5904 5963
ATGAGTTTCAATCGGACGAATTGTAGTAGCACAAATTCGCGTCGCTACCGTCTGTGTAGA
CCATGGGTGGCACTCGCGACAGGTGTCTGTCTGTATCTTATCGAGTTATATGTTGAGCTG
5964 6023
GGTACCCACCGTGAGCGCTGTCCACAGACAGACATAGAATAGCTCAATATACAACTCGAC
CCTCCAGCAATCATGCAGGATGAGTCGCTATTGACGACCCTCTGCCTCGTAGGCCCATCC
6024 6083
GGAGGTCGTTAGTACGTCCTACTCAGCGATAACTGCTGGGAGACGGAGCATCCGGGTAGG
ATTGGTGGGCTTCCGACCCCTGCAACCCTACCCAGTGTCTTTTTCAGAGGAATGTCCGAC
6084 6143
TAACCACCCGAAGGCTGGGGACGTTGGGATGGGTCACAGAAAAAGTCTCCTTACAGGCTG
CCACTGCCCTTTCAGCTAGCACTCTTGCAGACCCTCATTAAGACGACAGGGGTGACCTGT
6144 6203
GGTGACGGGAAAGTCGATCGTGAGAACGTCTGGGAGTAATTCTGCTGTCCCCACTGGACA
AGCTTGGTGAATCGTGTGGTCAAGTTACGGATAGCACCCTATCCAGACTGGCTCTCTCTA
6204 6263
TCGAACCACTTAGCACACCAGTTCAATGCCTATCGTGGGATAGGTCTGACCGAGAGAGAT
GTGACTGACCCGACCTCACTCAACATTGCCCAAGTGTACCGGCCAGAACGTCAGATCAGG
6264 6323
CACTGACTGGGCTGGAGTGAGTTGTAACGGGTTCACATGGCCGGTCTTGCAGTCTAGTCC
AGGTGGATTGAGGAAGCGATAGCGACAAGCTCACACTCGTCACGCATAGCAACTTTCTTC
6324 6383
TCCACCTAACTCCTTCGCTATCGCTGTTCGAGTGTGAGCAGTGCGTATCGTTGAAAGAAG
CAGCAGCCCCTCACGGAGATGGCTCAGTTGCTTGCGAGGGACCTTTCAACAATGATGCCT
6384 6443
GTCGTCGGGGAGTGCCTCTACCGAGTCAACGAACGCTCCCTGGAAAGTTGTTACTACGGA
CTTCGACCCCGGGATATGTCGGCCTTATTCGCATTATCAAATGTCGCATACGGTTTAAGC
6444 6503
GAAGCTGGGGCCCTATACAGCCGGAATAAGCGTAATAGTTTACAGCGTATGCCAAATTCG

FIG. 18(4)

```
6504 ATTATAGATCTATTTCAAAAATCCTCTACCGTTGTTTCTGCAAGTCAAGCTGTCCATATC 6563
     ------+---------+---------+---------+---------+---------+---
     TAATATCTAGATAAAGTTTTTAGGAGATGGCAACAAAGACGTTCAGTTCGACAGGTATAG

6564 GAGGATGTTGCCCTAGAGAGTGTAAGGTATAAGGAATCTATCATCCAGGGTCTGTTAGAC 6623
     ------+---------+---------+---------+---------+---------+---
     CTCCTACAACGGGATCTCTCACATTCCATATTCCTTAGATAGTAGGTCCCAGACAATCTG

6624 ACCACTGAGGGGTATAACATGCAACCTTATTTGGAAGGTTGCACTTACCTTGCAGCCAAA 6683
     ------+---------+---------+---------+---------+---------+---
     TGGTGACTCCCCATATTGTACGTTGGAATAAACCTTCCAACGTGAATGGAACGTCGGTTT

6684 CAGTTACGTAGGTTGACATGGGGTCGAGACCTAGTTGGAGTCACAATGCCGTTTGTTGCC 6743
     ------+---------+---------+---------+---------+---------+---
     GTCAATGCATCCAACTGTACCCCAGCTCTGGATCAACCTCAGTGTTACGGCAAACAACGG

6744 GAGCAATTCCATCCTCACAGTTCTGTGGGTGCAAAGGCGGAACTCTACCTCGACGCTATT 6803
     ------+---------+---------+---------+---------+---------+---
     CTCGTTAAGGTAGGAGTGTCAAGACACCCACGTTTCCGCCTTGAGATGGAGCTGCGATAA

6804 ATATACTGCCCACAGGAGACATTGCGGTCACACCATCTGACTACCAGGGGGGACCAGCCG 6863
     ------+---------+---------+---------+---------+---------+---
     TATATGACGGGTGTCCTCTGTAACGCCAGTGTGGTAGACTGATGGTCCCCCCTGGTCGGC

6864 CTTTACCTCGGATCCAATACGGCTGTCAAGGTCCAGCGAGGTGAGATCACGGGCCTAACA 6923
     ------+---------+---------+---------+---------+---------+---
     GAAATGGAGCCTAGGTTATGCCGACAGTTCCAGGTCGCTCCACTCTAGTGCCCGGATTGT

6924 AAGTCAAGGGCTGCAAATCTAGTCAGGGACACTCTCGTTCTCCATCAGTGGTATAAAGTC 6983
     ------+---------+---------+---------+---------+---------+---
     TTCAGTTCCCGACGTTTAGATCAGTCCCTGTGAGAGCAAGAGGTAGTCACCATATTTCAG

6984 CGTAAAGTTACCGATCCACACTTGAACACCCTCATGGCACGCTTCTTACTTGAGAAGGGG 7043
     ------+---------+---------+---------+---------+---------+---
     GCATTTCAATGGCTAGGTGTGAACTTGTGGGAGTACCGTGCGAAGAATGAACTCTTCCCC

7044 TACACATCTGACGCTCGACCTAGCATCCAGGGTGGGACCCTCACGCATCGTCTCCCATCC 7103
     ------+---------+---------+---------+---------+---------+---
     ATGTGTAGACTGCGAGCTGGATCGTAGGTCCCACCCTGGGAGTGCGTAGCAGAGGGTAGG

7104 CGCGGAGACTCACGGCAGGGGCTTACTGGGTATGTAAATATACTAAGTACGTGGCTTCGA 7163
     ------+---------+---------+---------+---------+---------+---
     GCGCCTCTGAGTGCCGTCCCCGAATGACCCATACATTTATATGATTCATGCACCGAAGCT

7164 TTCTCAAGTGATTATCTTCACTCTTTCTCGAAATCATCAGACGACTATACAATCCACTTT 7223
     ------+---------+---------+---------+---------+---------+---
     AAGAGTTCACTAATAGAAGTGAGAAAGAGCTTTAGTAGTCTGCTGATATGTTAGGTGAAA

7224 CAGCATGTATTCACATACGGTTGCCTCTATGCTGATTCGGTGATTAGATCGGGCGGTGTT 7283
     ------+---------+---------+---------+---------+---------+---
     GTCGTACATAAGTGTATGCCAACGGAGATACGACTAAGCCACTAATCTAGCCCGCCACAA

7284 ATTTCCACTCCTTACCTTTTGAGTGCAAGTTGTAAAACATGCTTTGAGAAGATAGACTCA 7343
     ------+---------+---------+---------+---------+---------+---
     TAAAGGTGAGGAATGGAAAACTCACGTTCAACATTTTGTACGAAACTCTTCTATCTGAGT

7344 GAGGAGTTCGTCCTGGCATGTGAACCCCAATACAGGGGTGCTGAGTGGCTGATATCAAAG 7403
     ------+---------+---------+---------+---------+---------+---
     CTCCTCAAGCAGGACCGTACACTTGGGGTTATGTCCCCACGACTCACCGACTATAGTTTC

7404 CCAGTCACTGTCCCTGAGCAGATAACTGATGCTGAAGTCGAGTTTGACCCCTGTGTGAGT 7463
     ------+---------+---------+---------+---------+---------+---
     GGTCAGTGACAGGGACTCGTCTATTGACTACGACTTCAGCTCAAACTGGGGACACACTCA
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FIG. 18(5)

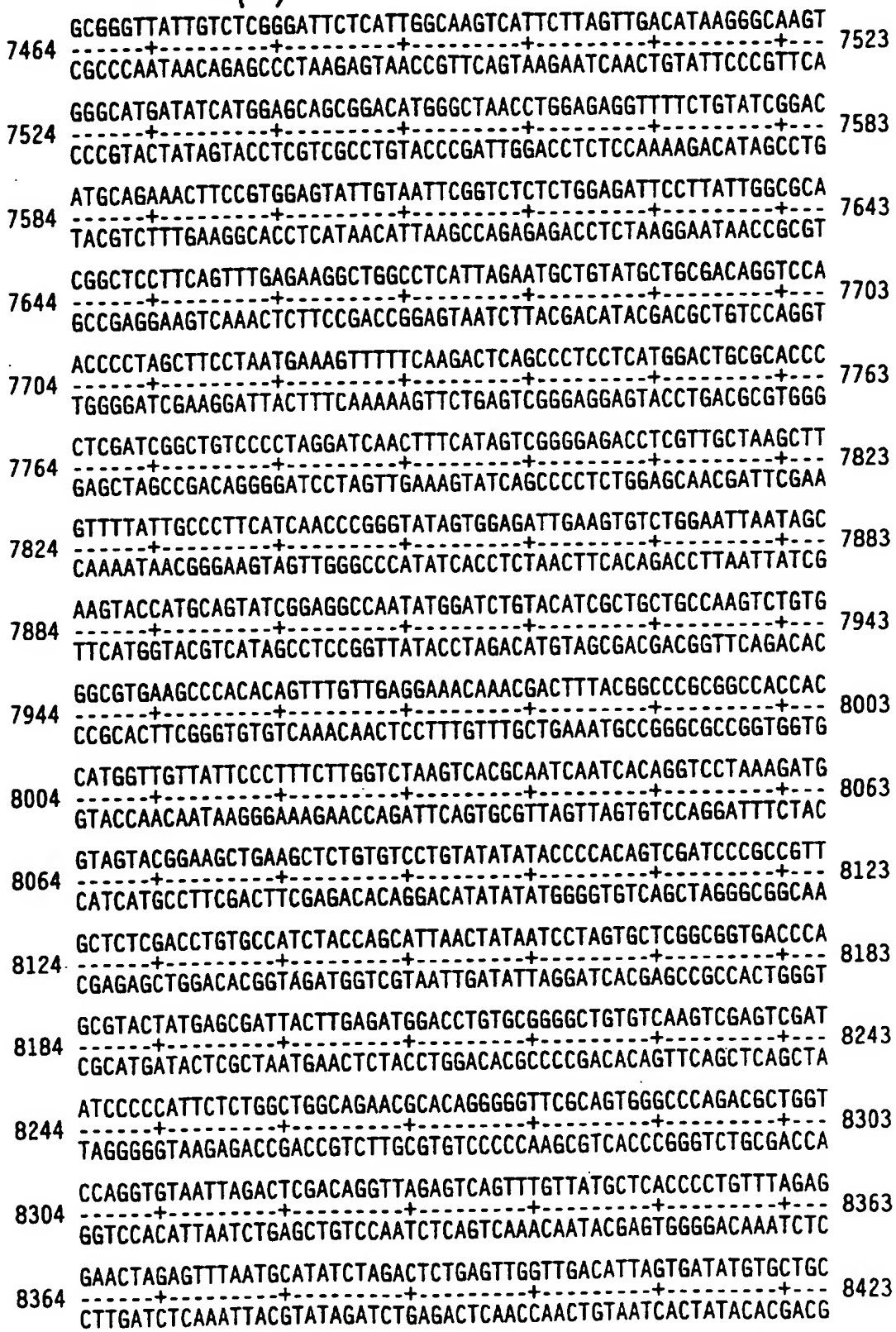

7464 GCGGGTTATTGTCTCGGGATTCTCATTGGCAAGTCATTCTTAGTTGACATAAGGGCAAGT CGCCCAATAACAGAGCCCTAAGAGTAACCGTTCAGTAAGAATCAACTGTATTCCCGTTCA 7523
7524 GGGCATGATATCATGGAGCAGCGGACATGGGCTAACCTGGAGAGGTTTTCTGTATCGGAC CCCGTACTATAGTACCTCGTCGCCTGTACCCGATTGGACCTCTCCAAAAGACATAGCCTG 7583
7584 ATGCAGAAACTTCCGTGGAGTATTGTAATTCGGTCTCTCTGGAGATTCCTTATTGGCGCA TACGTCTTTGAAGGCACCTCATAACATTAAGCCAGAGAGACCTCTAAGGAATAACCGCGT 7643
7644 CGGCTCCTTCAGTTTGAGAAGGCTGGCCTCATTAGAATGCTGTATGCTGCGACAGGTCCA GCCGAGGAAGTCAAACTCTTCCGACCGGAGTAATCTTACGACATACGACGCTGTCCAGGT 7703
7704 ACCCCTAGCTTCCTAATGAAAGTTTTTCAAGACTCAGCCCTCCTCATGGACTGCGCACCC TGGGGATCGAAGGATTACTTTCAAAAAGTTCTGAGTCGGGAGGAGTACCTGACGCGTGGG 7763
7764 CTCGATCGGCTGTCCCCTAGGATCAACTTTCATAGTCGGGGAGACCTCGTTGCTAAGCTT GAGCTAGCCGACAGGGGATCCTAGTTGAAAGTATCAGCCCCTCTGGAGCAACGATTCGAA 7823
7824 GTTTTATTGCCCTTCATCAACCCGGGTATAGTGGAGATTGAAGTGTCTGGAATTAATAGC CAAAATAACGGGAAGTAGTTGGGCCCATATCACCTCTAACTTCACAGACCTTAATTATCG 7883
7884 AAGTACCATGCAGTATCGGAGGCCAATATGGATCTGTACATCGCTGCTGCCAAGTCTGTG TTCATGGTACGTCATAGCCTCCGGTTATACCTAGACATGTAGCGACGACGGTTCAGACAC 7943
7944 GGCGTGAAGCCCACACAGTTTGTTGAGGAAACAAACGACTTTACGGCCCGCGGCCACCAC CCGCACTTCGGGTGTGTCAAACAACTCCTTTGTTTGCTGAAATGCCGGGCGCCGGTGGTG 8003
8004 CATGGTTGTTATTCCCTTTCTTGGTCTAAGTCACGCAATCAATCACAGGTCCTAAAGATG GTACCAACAATAAGGGAAAGAACCAGATTCAGTGCGTTAGTTAGTGTCCAGGATTTCTAC 8063
8064 GTAGTACGGAAGCTGAAGCTCTGTGTCCTGTATATATACCCCACAGTCGATCCCGCCGTT CATCATGCCTTCGACTTCGAGACACAGGACATATATATGGGGTGTCAGCTAGGGCGGCAA 8123
8124 GCTCTCGACCTGTGCCATCTACCAGCATTAACTATAATCCTAGTGCTCGGCGGTGACCCA CGAGAGCTGGACACGGTAGATGGTCGTAATTGATATTAGGATCACGAGCCGCCACTGGGT 8183
8184 GCGTACTATGAGCGATTACTTGAGATGGACCTGTGCGGGGCTGTGTCAAGTCGAGTCGAT CGCATGATACTCGCTAATGAACTCTACCTGGACACGCCCCGACACAGTTCAGCTCAGCTA 8243
8244 ATCCCCCATTCTCTGGCTGGCAGAACGCACAGGGGGTTCGCAGTGGGCCCAGACGCTGGT TAGGGGGTAAGAGACCGACCGTCTTGCGTGTCCCCCAAGCGTCACCCGGGTCTGCGACCA 8303
8304 CCAGGTGTAATTAGACTCGACAGGTTAGAGTCAGTTTGTTATGCTCACCCCTGTTTAGAG GGTCCACATTAATCTGAGCTGTCCAATCTCAGTCAAACAATACGAGTGGGGACAAATCTC 8363
8364 GAACTAGAGTTTAATGCATATCTAGACTCTGAGTTGGTTGACATTAGTGATATGTGCTGC CTTGATCTCAAATTACGTATAGATCTGAGACTCAACCAACTGTAATCACTATACACGACG 8423

FIG. 18(6)

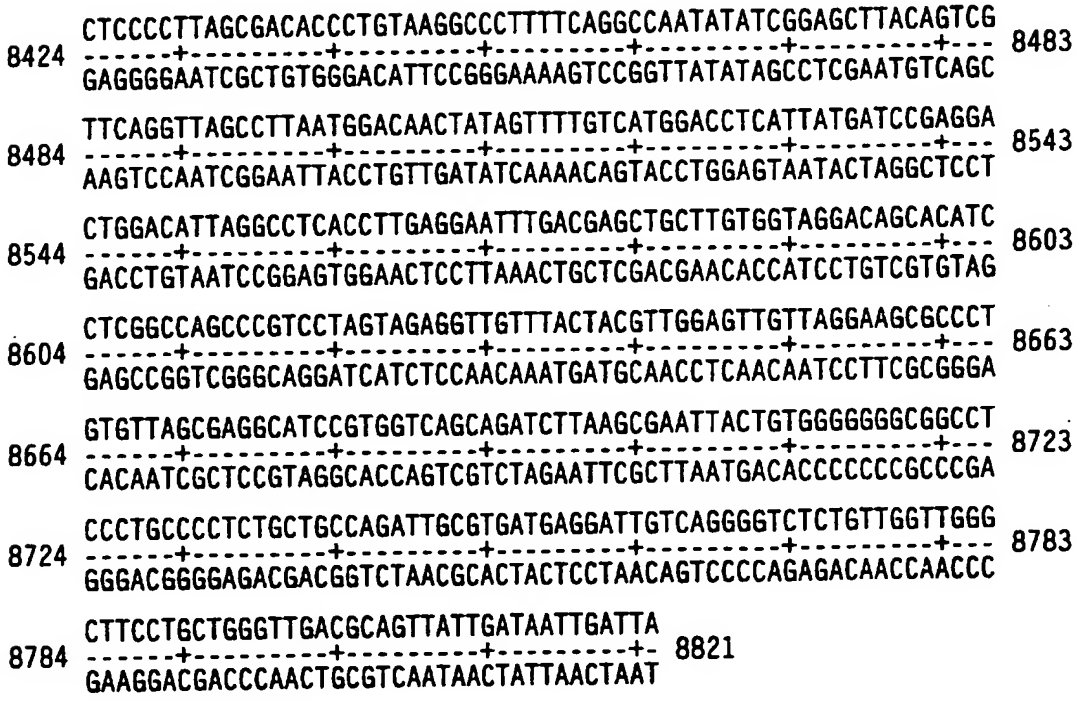

8424 CTCCCCTTAGCGACACCCTGTAAGGCCCTTTTCAGGCCAATATATCGGAGCTTACAGTCG
GAGGGGAATCGCTGTGGGACATTCCGGGAAAAGTCCGGTTATATAGCCTCGAATGTCAGC 8483
8484 TTCAGGTTAGCCTTAATGGACAACTATAGTTTTGTCATGGACCTCATTATGATCCGAGGA
AAGTCCAATCGGAATTACCTGTTGATATCAAAACAGTACCTGGAGTAATACTAGGCTCCT 8543
8544 CTGGACATTAGGCCTCACCTTGAGGAATTTGACGAGCTGCTTGTGGTAGGACAGCACATC
GACCTGTAATCCGGAGTGGAACTCCTTAAACTGCTCGACGAACACCATCCTGTCGTGTAG 8603
8604 CTCGGCCAGCCCGTCCTAGTAGAGGTTGTTTACTACGTTGGAGTTGTTAGGAAGCGCCCT
GAGCCGGTCGGGCAGGATCATCTCCAACAAATGATGCAACCTCAACAATCCTTCGCGGGA 8663
8664 GTGTTAGCGAGGCATCCGTGGTCAGCAGATCTTAAGCGAATTACTGTGGGGGGGCGGCCT
CACAATCGCTCCGTAGGCACCAGTCGTCTAGAATTCGCTTAATGACACCCCCCCGCCCGA 8723
8724 CCCTGCCCCTCTGCTGCCAGATTGCGTGATGAGGATTGTCAGGGGTCTCTGTTGGTTGGG
GGGACGGGGAGACGACGGTCTAACGCACTACTCCTAACAGTCCCCAGAGACAACCAACCC 8783
8784 CTTCCTGCTGGGTTGACGCAGTTATTGATAATTGATTA
GAAGGACGACCCAACTGCGTCAATAACTATTAACTAAT 8821

FIG. 19(1)

```
   1  GGTAGACCAG CTCCTGAAGA ACCTCAGGAA GAACCCCTCC ATGATCTCAG
  51  ACCCAGACCA GCGAACCGGA AGGGAGCAGC TATCGAATGA TGAGCTTATC
 101  AAGAAGCTAG TGACGGAGCT GGCCGAGAAT AGCATGATCG AGGCTGAGGA
 151  GGTGCGGGGC ACTCTTGGGG ACATCTCGGC TCGCATCGAG GCAGGGTTTG
 201  AGTCCCTGTC CGCCCTCCAA GTGGAAACCA TCCAGACAGC TCAGCGGTGC
 251  GACCACTCCG ATAGCATCAG AATCCTTGGC GAGAACATCA AGATACTGGA
 301  TCGCTCCATG AAGACAATGA TGGAGACAAT GAAGCTCATG ATGGAGAAGG
 351  TGGACCTCCT CTACGCATCA ACCGCCGTTG GGACCTCTGC ACCCATGTTG
 401  CCCTCCCATC CTGCACCTCC GCGCATTTAT CCCCAGCTCC CAAGTGCCCC
 451  GACAGCGGAT GAGTGGGACA TCATACCATA AAAAAATCGA ATCACCATGA
 501  ATTCAAAGCA TTCCTATGTG GAGCTCAAGG ACAAGGTAAT CGTCCCTGGA
 551  TGGCCCACAC TGATGCTTGA GATAGACTTT GTAGGAGGGA CTTCACGGAA
 601  CCAGTTCCTT AACATCCCAT TTCTTTCAGT GAAAGAGCCT CTGCAGCTTC
 651  CACGCGAGAA GAAGTTGACC GACTACTTCA CCATTGACGT AGAGCCAGCA
 701  GGTCATTCCC TGGTCAACAT ATACTTCCAG ATTGACGACT TCTTGCTCCT
 751  AACACTCAAC TCACTGTCCG TATACAAGGA CCCGATTAGG AAATACATGT
 801  TCCTACGCCT CAACAAGGAA CAGAGCAAGC ACGCAATTAA TGCAGCTTTC
 851  AATGTCTTCT CTTATCGGCT TCGGAACATT GGTGTTGGCC CTCTCGGCCC
 901  AGACATTCGA TCTTCAGGGC CTTAGTTGCA ATACTGACTC CACTCCTGGA
 951  TTAATCGATC TGGAGATAAG GCGACTTTGA CACACCCCAA CGGAAAATGT
1001  CATTTCATGC GAGGTTAGTT ATCTTAACCA CACGACTATT AGCCTCCCGG
1051  CAGTCCACAC GTCATGCCTC AAGTACCACT GCAAAACCTA TTGGGGATTC
1101  TTTGGTAGCT ACAGCGCTGA CCGAATCATC AATCGGTACA CTGGTACTGT
1151  TAAGGGTTGT TTAAACAACT CAGCGCCAGA GGATCCCTTC GAGTGCAACT
1201  GGTTCTACTG CTGCTCGGCG ATTACAACAG AGATCTGCCG ATGCTCTATT
1251  ACAAATGTCA CGGTGGCTGT ACAGACATTC CCACCGTTCA TGTACTGCAG
1301  TTTCGCGGAC TGTAGTACTG TGAGTCAGCA GGAGCTAGAG AGTGGAAAGG
1351  CAATGCTGAG CGATGGCAGT ACCTTAACTT ATACCCCGTA TATCTTACAA
1401  TCAGAAGTCG TGAACAAAAC CCTTAATGGG ACTATACTCT GCAACTCATC
1451  CTCCAAGATA GTTTCCTTCG ATGAATTTAG GCGTTCATAC TCCCTAGCGA
```

FIG. 19(2)

```
1501  ATGGTAGTTA CCAGAGCTCA TCAATCAATG TGACGTGTGT AAACTACACG
1551  TCGTCCTGCC GGTCCAAGTT GAGAAGGCGG CGTAGGGATA CTCAACAGAT
1601  TGAGTACCTA GTTCACAAGC TTAGGCCTAC ACTGAAAGAT GCGTGGGAGG
1651  ACTGTGAGAT CCTCCAGTCT CTGCTCCTAG GGGTGTTTGG TACTGGGATT
1701  GCAAGTGCTT CGCAATTCTT GAGGGGCTGG CTCAACCACC CTGATATCAT
1751  CGGGTATATA GTTAATGGAG TTGGGGTAGT CTGGCAATGC CATCGTGTTG
1801  ATGTCACGTT CATGGCGTGG AATGAGTCCA CATATTACCC TCCAGTAGAT
1851  TACAATGGAC GGAAGTACTT TCTGAATGAT GAGGGGAGGC TACAAACAAA
1901  CACCCCCGAG GCAAGGCCAG GGCTTAAGCG GGTCATGTGG TTCGGCAGGT
1951  ACTTCCTAGG GACAGTAGGG TCTGGGGTGA AACCGAGGAG GATTCGGTAC
2001  AATAAGACCT CACATGATTA CCATCTAGAG GAGTTTGAGG CAAGTCTCAA
2051  CATGACCCCC CAGACCAGTA TCGCCTCGGG TCATGAGACA GACCCCATAA
2101  ATCATGCCTA CGGAACGCAG GCTGACCTCC TTCCATACAC CAGGTCTAGT
2151  AATATAACGT CTACAGATAC AGGCTCAGGC TGGGTGCACA TCGGCCTACC
2201  CTCATTTGCT TTCCTCAATC CTCTCGGGTG GCTTAGGGAC CTACTTGCGT
2251  GGGCGGCCTG GTTGGGTGGG GTTCTATACT TAATAAGTCT TTGTGTTTCC
2301  TTACCAGCCT CCTTCGCGAG GAGGAGACGC CTCGGCCGGT GGCAGGAATA
2351  AACCGTACCG ACCAATCTCT TAAAAACCCT CTTCTCGGGA CAGAGGTCTC
2401  TTTCTGCCTT AAATCGAGTT CACTCCCCCA TCACGTACGA GCATTGGGCC
2451  AGATTAAAGC AAAGAACCTG GCATCCTGTG ACTATTACTT GCTATTCCGC
2501  CAAGTTGTAT TGCCCCCTGA AGTATATCCC ATTGGTGTCT TAATAAGAGC
2551  TGCGGAGGCC ATACTAACAG TTATAGTATC AGCTTGGAAG CTGGATCACA
2601  TGACAAAGAC CCTATACTCC TCTGTGAGAT ATGCACTCAC CAATCCCCGG
2651  GTCCGGGC
```

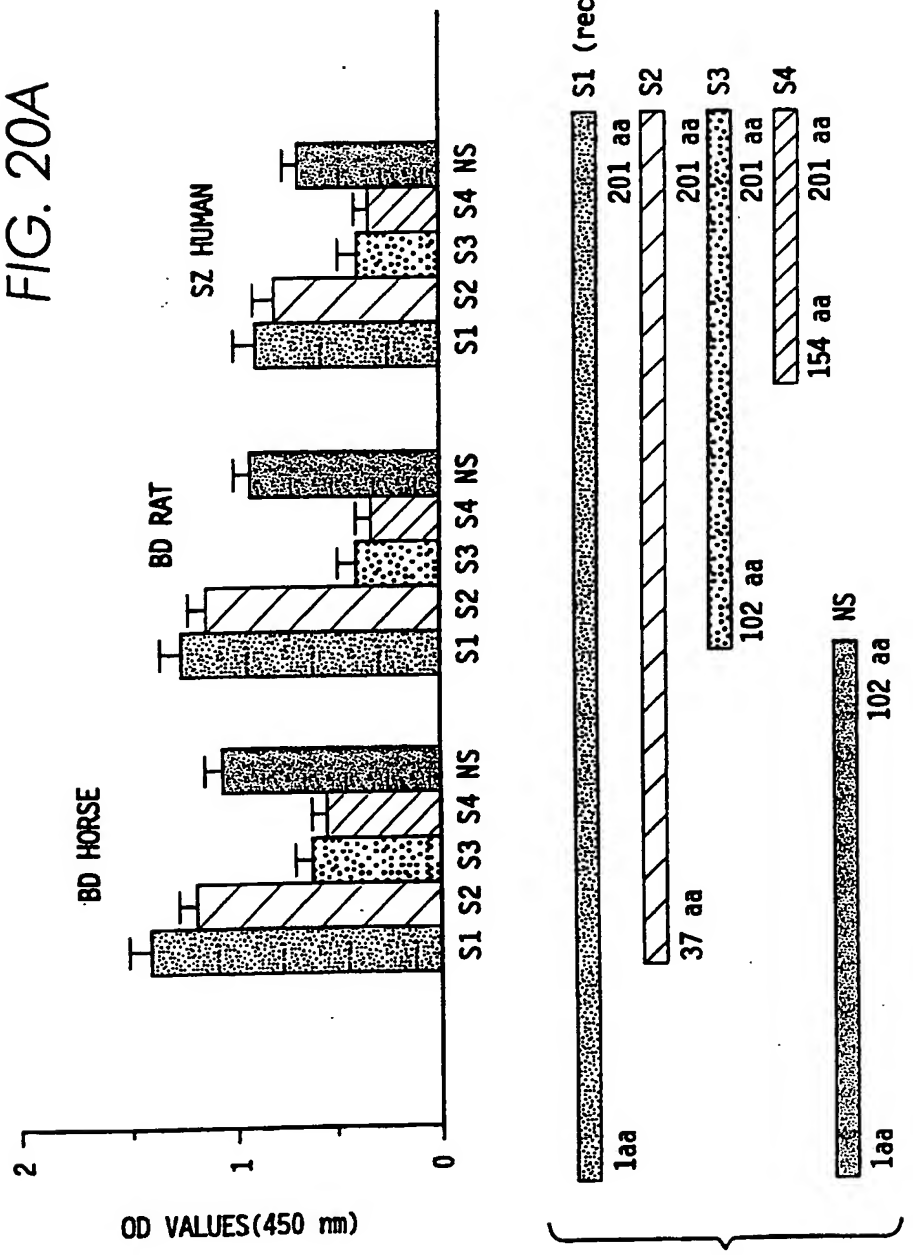
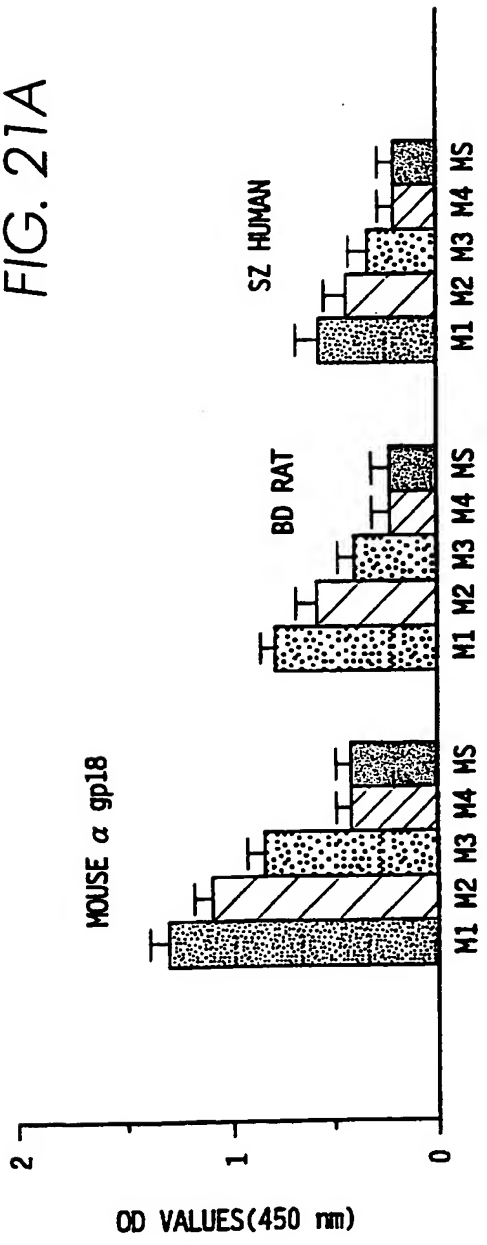


FIG. 20A

FIG. 20B

FIG. 21A



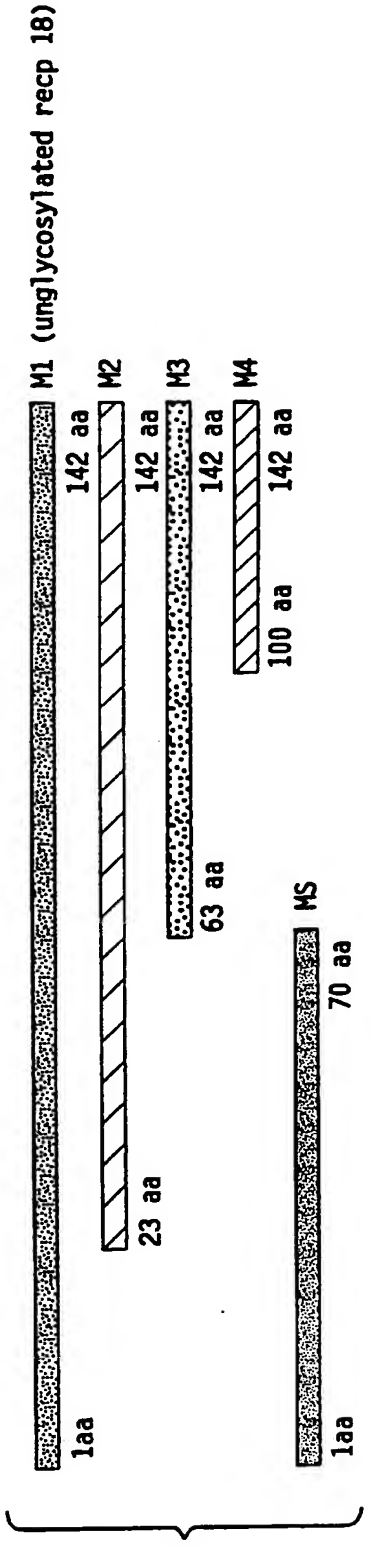


FIG. 21B

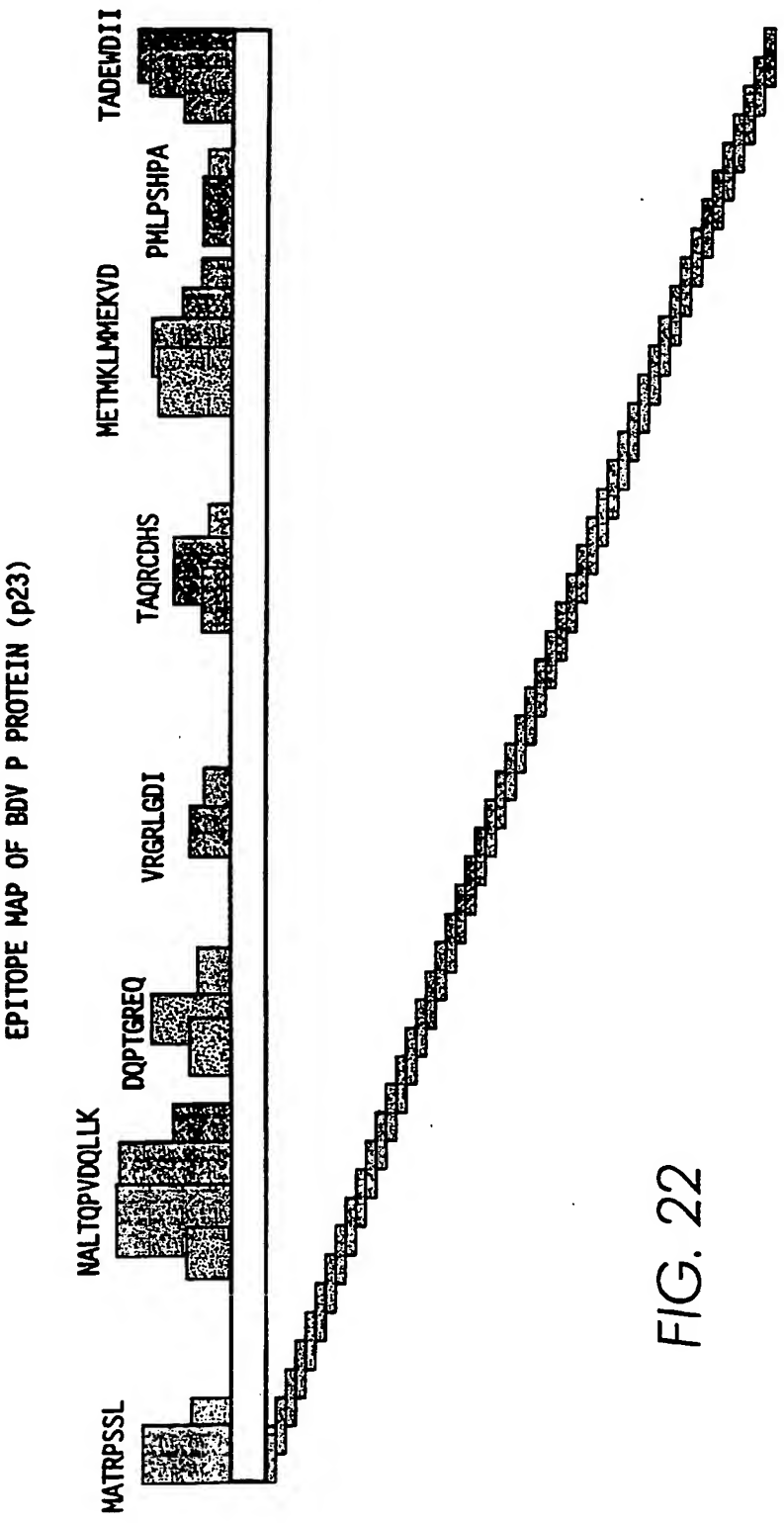

EPITOPE MAP OF BDV P PROTEIN (p23)
MATRPSSL
NALTQPVDQLLK
DQPTGREQ
VRGRLGDI
TAQRCDHS
METMKLMMEKVD
PMLPSHPA
TADEWDII

FIG. 22

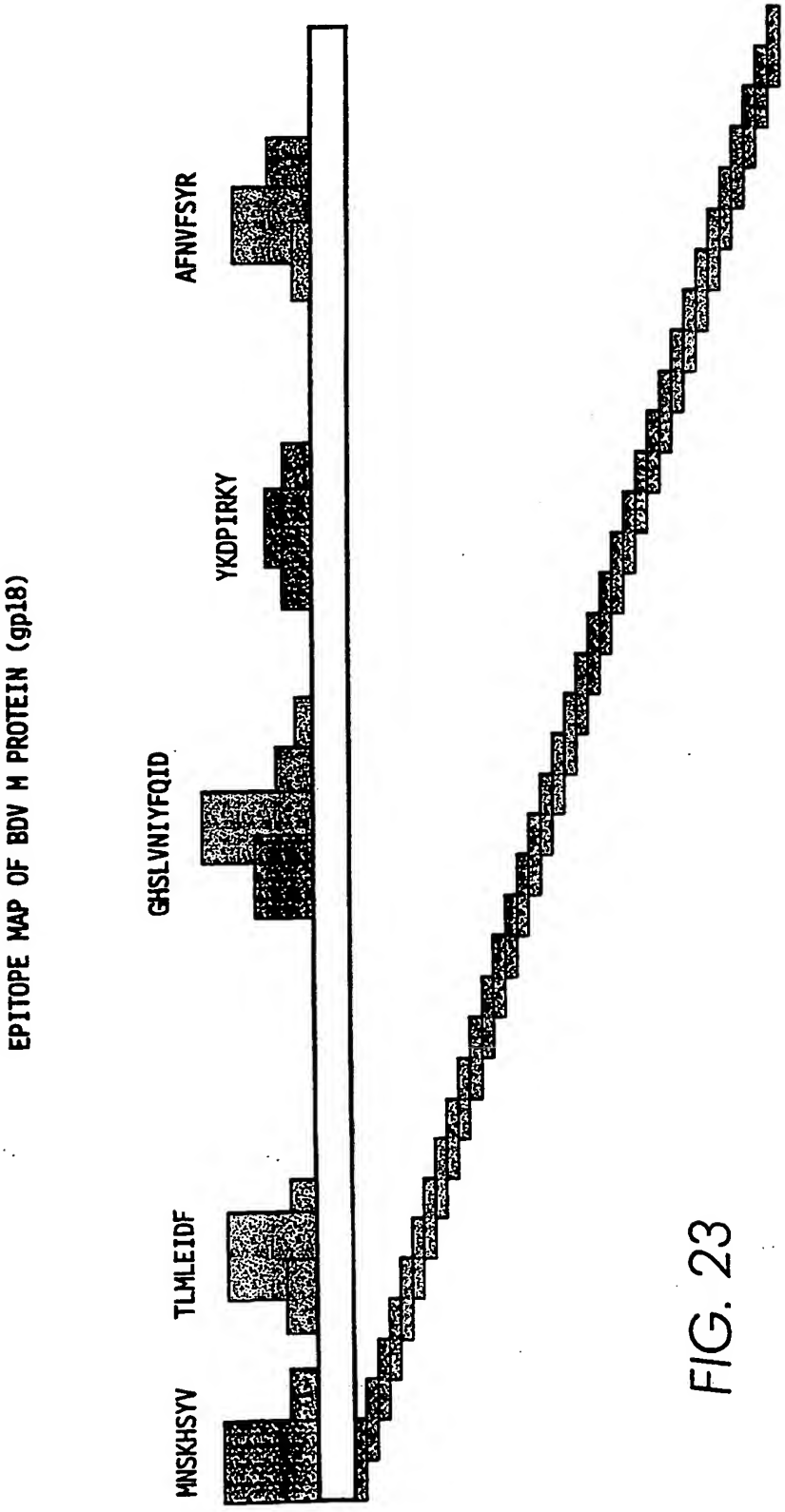

EPITOPE MAP OF BDV M PROTEIN (gp18)
MNSKHSYV
TLHLEIDF
GHSLVNIYFQID
YKDPIRKY
AFNVFSYR

FIG. 23

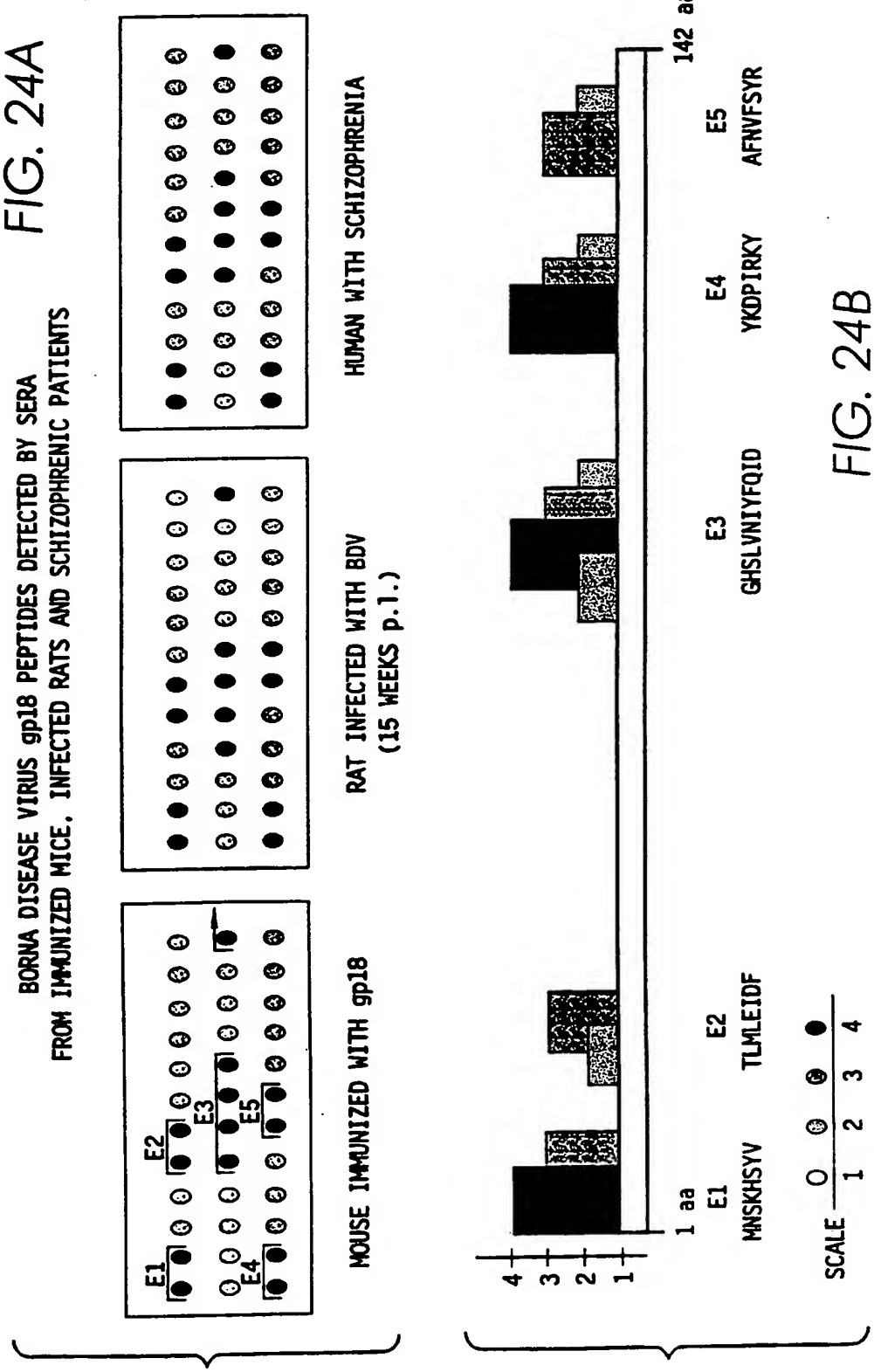


FIG. 24A

FIG. 24B

FIG. 25

```
  1 MQPSMSFLIGFGTLVLVLSARTFDLQGLSCNTDSTPGLIDLEIRRLCHTP
 51 TENVISCEVSYLNHTTISLPAVHTSCLKYHCKTYWGFFGSYSADRIINRY
101 TGTVKGCLNNSAPEDPFECNWFYCCSAITTEICRCSITNVTVAVQTFPPF
151 MYCSFADCSTVSQQELESGKAMLSDGSTLTYTPYILQSEVVNKTLNGTIL
201 CNSSSKIVSFDEFRRSYSLTNGSYQSSSINVTCANYTSSCRPRLKRRRRD
251 TQQIEYLVHKLRPTLKDAWEDCEILQSLLLGVFGTGIASASQFLRSWLNH
301 PDIIGYIVNGVGVVWQCHRVNVTFMAWNESTYYPPVDYNGRKYFLNDEGR
351 LQTNTPEARPGLKRVMWFGRYFLGTVGSGVKPRRIRYNKTSHDYHLEEFE
401 ASLNMTPQTSIASGHETDPINHAYGTQADLLPYTRSSNITSTDTGSGWVH
451 IGLPSFAFLNPLGWLRDLLAWAAWLGGVLYLISLCVSLPASFARRRRLGR
501 WQE
```